Clinical Grand Rounds at M.D. Anderson
The University of Texas MD Anderson Cancer Center
"Developing Novel Molecular Therapy for Head and Neck Cancer"*
Dr. Gary L. Clayman, MD
Director, Basic Science Research
Department of Head and Neck Surgery

It's a real honor to have the opportunity to present oncology grand rounds here at M.D. Anderson and I appreciate the introduction by Dr. Gefford and if I can have the first slide please. The title of this talk is developing novel molecular therapy for head and neck cancer. This doesn't necessarily just apply to head and neck cancer. We're using head and neck cancer as a model, but certainly my particular clinical interest has driven me towards this particular entity. There are several clinical problems, or clinical dilemmas that occur in the management of patients that have head and neck cancer. The particular head and neck cancer that we are focusing our research efforts on is squamous cell cancer of the upper digestive tract.

To just refresh those that are minimally or nonfamiliar, local regional control remains a major problem in patients afflicted with this cancer. With only 10-15% of patients dying of this disease alone. The local regional failure continues to remain a major problem. Second primary cancers occur in this patient population probably a rate approximating 4-7% a year. This is really more a problem in patients that have early stage disease. Those patients with advance stage disease tend to succumb to their own disease process. Dissecting the molecular cascade that's occurring in the progression of head and neck cancer is critical in understanding this process, and it's probably the most humbling thing with regard to this particular type of cancer is that we truly have not impacted upon the overall and disease specific survival in patients that are afflicted with this disease.

I'd just like to show you this slide. It was a study that we did here a couple of years ago that looked at patients that were undergoing organ preservation attempts in studies that were directed under the offices of Dr. Wang-Kay Hong, and in this study, patients with advanced hyperthyroidal or laryngeal cancers that would require larengectemy received chemotherapy and radiation therapy in attempts to preserve their organs. We therefore sought to determine whether these contemporary management how compared to our respective controls that had been treated in years gone past. As you can see in the disease specific survival, we do know better. These patients that had organ preservation attempts also had surgical salvage if they had failure. But basically stating this is just sort of a depiction that we do know better than we did 20-30 years ago.

There may be many different strategies for novel gene therapy in the management of solid organ tumors and I just gave a list of several of them here. One of them may be the delivery of a toxic gene or potentially a metabolite. The HSDTK gene if you've been reading the literature, is one that may represent this particular category. In that particular means of therapy, you would deliver a particular gene and then deliver either it itself would be toxic or something,

* Transcribed from a VCR tape of the Clinical Grand Rounds presentation of Dr. Clayman at the University of Texas MD Anderson Cancer Center. The VCR tape includes the figures that are referred to in this talk and can be made available upon request.

for example, with gancyclovir therapy HSDTK gene expression induces cell death. You may be able to augment immunogenicity of a tumor by gene therapy, by enhancing some particular cell membrane or characteristic of a tumor that can be monitored by the immune system or conversely we may be able to regulate the immune system either ex vivo or in vivo. You may be able to alter the chemosensitivity or radiation sensitivity of a tumor. In a recent publication by Dr. Wei-Wei Zhang and Roth et al. from this institution they showed that chemosensitivity may be augmented by a particular gene therapy. You may be able to reverse early molecular events in the carcinogenic cascade, and in this we're presently pursuing efforts that we're not going to talk about today, but you may be able to reverse the premalignant cascade in order to develop a prevention strategy in the management of this process. And, lastly, if you can target the particular cell cycle regulators or induce apoptosis in tumor cells, you may be able to regulate tumor cells by intervening in these steps.

We're particularly attracted to a particular kind of gene therapy or molecular vector that's an adenovirus vector. One of the attractions of this is its natural tropism for zero digestive tract epithelium and here we hope that we could adequately transduce the cells of interest. In other organ tissue systems, high transduction efficiency has been shown with these particular vectors and importantly if you delete particular components of the adenovirus and in particular E1A and E1B components you can render it replication defective and in this manner you can obviate some of the problems with permanent integration of extraneous components into the genome.

In our initial experiments we thought to determine whether we could simply transduce cells. And here we used a particular marker and this marker is the β-galactosidase marker. It's a β-galactosidase on an adenovirus replication defective vector. And in a dose response experiment, we sought to determine the transduction efficacy in squamous cell cancer cell lines. Here was have a mock infected cell line where we see no β-galactosidase expression and here we see at a 50 multiplicity of infection we see simply 50 viral particles per tumor cell, we see approximately 40-50% transduction efficiency. With higher viral titers here 100 multiplicity of infection, we see essentially our maximal infection rate of about 60-65%. With repeat infection, we see 100% transduction of these tumor cells. When we graphically depict this, independent of the tumor cell line and we plateau here this is with a single infection 24 hours after infection, our β-galactosidase expression is approximately 65-70%. Dr. Wei-Wei Zhang and Dr. Roth of this institution have collaborated with us in these studies. Drs. Wei-Wei Zhang and Roth have developed a wild-type p53 virus vector. Here in a linear diagram for you is the construction schematic for CMV promoter sites, the wild-type p53 cDNA and a polyadenylation site and the cell is replication defective at A1A in a component of B1B deletion.

Here are initial experiments that we sought to determine with this particular adenovirus vector that Dr. Roth generously gave to us so that we could induce mRNA expression in squamous cell head and neck cell lines. In these experiments the 293 packaging cell line that the virus is propagated in we see the positive control. In our negative control we see no expression of the p53 MRNA. In these experiments we used the parental cell line and we use a replication defective virus without any particular gene or promoter attached and then the wild-type p53 adenovirus vector. And here we see a small induction of endogenous but a very significant exogenous mRNA expression replication defective virus and the parental cell line controls with no increased expression. And again this was independent of the cell line that's utilized parental

control replication defective virus and here the infected cell line. Message is induced and we hope that protein would be induced also here in Western blot experiments with the same type of experimental schematic for packaging cell lines with significant expression of the p53 protein here the parental cell lines in very low levels of p53 protein. Both of the cell lines by the way are mutated for p53. This cell line treated with replication defective virus and here the adenovirus infected with the p53 and a very significant induction of p53 protein. Again, independent of the cell line that was utilized. From a light microscopic standpoint we sought to using immunohistochemistry to verify this protein expression as well as our transduction efficacy as we saw with the β-galactosidase virus. Here, the mock infected control, this is again, this is a mutated p53 cell line, here infected with the adenovirus p53 we see the characteristic nuclear staining that we would expect to see with the exogenous p53 expression. Again, the transduction efficiency approximates that that we saw with the β-galactosidase adenovirus expression vector.

Dr. Wei-Wei Zhang performed these experiments in order to determine the duration of this transient expression of p53 in infected cells. Here in a time course experiment over 15 days essentially no p53 is expressed two weeks following this transient expression with the p53 adenovirus vector.

In in vitro growth curve experiments we then sought to determine the effects on in vitro cell growth. Here is a mutated p53 cell line, we use mock infection in order to determine a routine growth rate in a logarithmic growth rate is seen. **Here, a replication defective virus control seeing that essentially no significant difference in growth rate and then treated with the p53 the wild type p53 adenovirus we see essentially total cell death within three days in the cell line. This is not dependant upon this particular cell line. These are mutated p53 cell lines. These are wild type p53 cell lines treated with wild-type p53 adenovirus. Here mock infection replication defective virus. Here the adenovirus p53 treatment independent of cell line and here even though wild type endogenous p53 the cells although they have a little bit delay in their cell death, the cell death occurred within 3-4 days following transient infection.** In nonmalignant cells, we sought to determine what this infection would produce. In fibroblast cell lines it has been established in laboratories. Here we have the mock infection in white; in green the replication defective virus and here the p53 adenovirus infection. The growth rate remains unchanged. Fibroblasts have nearly 100% transduction efficiency as compared to the cell lines and they express these infections without any difficulty. We sought to determine what may be the potential mechanism of cell loss that we were exhibiting in our in vitro growth assays. Here, in a DNA fragmentation experiment we have fragments and one hundred base pair ladder here. Here is mock infection. These are 24 hours after infection with the particular therapy. Mock infection a replication defective virus we see no evidence of any fragmentation and here in a time curve experiment have 4 hours, 8 hours, 22 hours you see the induction of fragmentation and at 30 hours. That is consistent with apoptosis death. Further studies are being done with regard to DNA labeling as well as electron microscopy in order to confirm this as the mechanism of in vitro as well as in vivo cell death that I'll show you in just a couple of minutes.

We then sought to determine in vivo model how we could develop novel molecular therapy in the management of this process. We have developed a flap model in a nude mouse. The flap model may use from one flap up to 4 flaps in these animals, depending on the particular experiments that I will describe to you later. A small incision is made in a flap that is elevated in

the subcutaneous plain and tumor cells are delivered into the flap and pipetted into the flap in order to deliver a particular number of tumor cells and also not to extravate the tumor cells outside of the pocket site. The tumor cells are held in place with a horizontal matra-suture that does not allow extravasations of any fluids that are delivered into the pocket. Then, the central therapeutic intervention is delivered. We have done experiments from 2 days later to up to 5 days later to deliver the novel therapy. In these earlier experiments, we did 3 flap models and in this flap we developed a flap, delivered the tumor, and then waited 3 or 4 days later in order to establish a subcutaneous nodule tumor. At that time, in these experiments, these were three days following this tumor, we elevated up the flap, isolated the nodule and then delivered the potential therapy. In the anterior flap here we delivered p53 adenovirus vector. In the posterior flap here we delivered replication defective virus. In the left posterior flank we delivered mock infection with phosphate buffered saline. We then allowed these animals to grow and allowed the tumor sites to grow. And as you see significant inhibition of tumor cell growth as compared to replication defective virus in the mock infection control. In a graphic sense, here in table format, the replication defective virus here has transport media alone which was PBS and the p53 adenovirus treated here and we see significant suppression as compared to replication defective virus through transport media alone independent of cell line utilized. And in these animals a slower growth curve. Two of these 6 animals had total suppression or total regression of the tumor both clinically as well as histologically when the flap model was evaluated histopathologically.

I enjoy Gary Larson and his depiction of potential laboratory peer pressure. One of the clinical dilemmas that we occur with in head and neck cancer is patients frequently develop or present with advance stage disease and one of the most frequently presented signs as well as symptoms is the development of the neck mass. Patients who develop the neck mass that is clinically palpable or greater than 2 cm in size, greater than 50% of them will have microscopic residual disease after aggressive surgical management of this process. To show you management of a process as depicted in the previous slide, here are a modified neck dissection is performed with the removal of the fibrolymphatic and facial contents of the superficial and deep compartments of the neck, major vascular, neural and muscular structures are spared from microscopic residual disease remains within this neck in the majority of our patients. In contemporary medicine today management of microscopic residual disease is performed with external beam radiation therapy. The results I've already described to you with advance stage disease. We continue to have major control problems in local and regional disease. This is a potential model for the delivery of novel intervention.

At this time, the entire environment for microscopic residual disease is available for intervention. How can we potentially predict those patients that have microscopic residual disease? We know that patients that develop neck masses greater than 2 cm in size, greater than 50% of these patients by the work of Barnes et al. of the University of Pittsburgh, have extracapsular or microscopic residual disease in the neck. The more radical the surgery does not change that process. As this size increases, the percentage approaches nearly 100%. Patients that present with neurotropic symptoms of their cancers, for example, diskinesia or akinesia of the cranial nerve or paralysis of the cranial nerve almost 100% of them will have microscopic residual disease that will require additional therapy in order to manage their process. Patients of the head and neck have a particular process that may induce field carcinogenesis that has been

proposed and propagated by many investigators within and outside of this institution. Those of our patients that have diffuse mucosal diseases that have erythroleukoplatic changes throughout their digestive tract, we frequently are fraught with chasing these margins from a surgical as well as from other intervention standpoints. Those patients that have deeply infiltrated tumors frequently have microscopic residual disease also.

We sought to develop a model for microscopic residual disease and novel intervention in this type of a model. In this model, we sought to determine transduction efficiency in our flap model that I previously described for you. In this model, we elevate the flap as I described to you before and then with our intervention I delivered mock infection or here the β-galactosidase adenovirus expression vector to determine simply transduction efficiency. Here mock infection we see no evidence of any ex-gal expression. Here with 10^6 plaque forming units being delivered into this environment we see a small amount of expression of the β-galactosidase. Here at 10^7 there essentially a log phase increase viral dose. We see more diffuse expression of the β-galactosidase and some inflammatory or edematous changes here. And here at 1 log dose even higher diffuse expression throughout multiple layers including the subdermal as well as muscular layer. Again, some edematous changes are also seen in this pocket that may be result of viral toxicity.

Utilizing the adenovirus p53 vector we then sought to establish small tumors within the pockets and then deliver the adenovirus vector. These are microscopic tumors. They're not clinically palpable at the time of intervention. This is a wild type tumor cell line that we utilize in order to make this more demonstrable for you today. Here to orient you is the dermal components and then the underlying tissues. The tumor here is established. This is treated with mock infection with phosphate buffered saline and then this is immunostained with p53 antibody that will detect both mutated and wild type p53. Here no significant expression of p53 protein. Here again to orient you, the overlying skin, the pocket of tumor, here treated with 10^6 plaque forming units of the wild type p53 adenovirus we see expression of the p53 proteins within the tumor. We see some peripheral necrosis but you certainly do see some viable tumor there in this pocket. At one log dose higher here we see the overlying skin. Here we see diffuse expression of the p53 protein from the muscular layer beneath to the subdermal area there is no evidence of any viable tumor and here with our highest viral dosing at 10^9 plaque forming units the overlying skin again to orient you some artifact here on sectioning but we see total vacuolization of the tumor. We see diffuse expression of the p53 protein and we see some inflammatory cells within the surrounding areas. Probably as a result of viral toxicity.

What happens if we allow these animals to continue to grow? Here in animal experiments and clearly here is the wild type p53 adenovirus vector treatment site replication defective virus and here control phosphate buffered saline alone in these animals treated with 10^8 plaque forming units, no tumors developed. Here the respective control sites. These animals will essentially have to be sacrificed because of outgrowth of this, no tumor has developed. Dose response experiments were done with animals in the four flap models that I showed you before as well as individual animals. I used the individual animals here in order to depict this for you. Here at 10^9 plaque forming units for the tumor burden of 2.5×10^6 cells no tumor will ever develop. 10^8 plaque forming unit no tumor will ever develop in this particular cell line. At 10^7

plaque forming unit a small tumor will develop and will eventually outgrow the animal will succumb to this and here with a mock infection control the tumor growth.

In a table format we did multiple experiments. Both of these are mutated p53 cell lines. **These are wild type p53 cell lines and here what we see with the microscopic residual disease model treated with 2 ½ million cells that those mutated cell lines, none of them will ever develop tumors treated with 10^8 plaque forming units and this particular wild type p53 parental cell line none will develop tumors, but in this particular cell line it also has HPV positive, 2 of the 6 will develop tumors treated with 10^8 plaque forming units at 10^9 plaque forming units we have never seen tumor outgrowth.** These results are very intriguing and exciting to us and therefore sought to determine what effects they may have on markedly gross residual disease. In these experiments we established subcutaneous nodules within these animals and then allowed these nodules to grow to a tumor burden of greater than one cm. In this experiment we used the β-galactosidase adenovirus vector in order to establish transduction efficiency in a large established tumor. This tumor was treated with a single injection of β-galactosidase adenovirus in 100 microliter aliquot. It was injected and this is the artifact or the vacuolization that occurred in the area of the injection, but their diffuse expression of the β-galactosidase adenovirus within this established tumor. This is not limited to a single cell layer. For your orientation, this is a 63 fold magnification. Please ignore the anterior component of these animals, but in the posterior flanks these were treated with replication defective virus. These were treated with the wild type p53 adenovirus. We treated them with a schema for gross tumor. In this schema these 10^9 plaque forming units on a three times weekly basis for two consecutive weeks. **Following two consecutive weeks in every animal tested, we had greater than 50% reduction in the tumors.** There is some overlying dermal loss here with ulceration here with the replication defective virus in both of these two sites this also occurred.

To summarize our investigation to this point in time, we are excited about the results of p53 adenovirus as it effectively inhibits the implanted tumors of squamous cell carcinoma, cell lines of the head and neck. This happens in vitro as well as in vivo. Tumor growth in established tumors is inhibited in a dose dependant fashion. We can titrate this upon the number of tumor cells that we implant into the model. Basically, we know that our squamous cell head and neck tumor cell lines will acquire approximately 1 million cells in order to grow in nude mice. We can give them tumor burdens up to 5×10^6 cells or five million cells and effectively inhibit these with adequate transduction with p53 adenovirus. In time course experiments we know that the intervention needs to be established before there is gross palpable disease otherwise repeat treatment is required. **p53 adenovirus is effective in growth inhibition in squamous cancers of the head and neck in these in vitro as well as in vivo animal models independent of the parental cell lines p53 status.**

I have to tell you that this is unique to the squamous cell cancer of the head and neck. In Dr. Roth and Wei-Wei Zhang's work, this has not been shown in the non-small cell lung cancer model. What is indigenous to squamous cell cancer to the head and neck that makes these transformed cells responsive to this particular therapy unlike other solid organ tissue systems that have been established have not been clearly elucidated at this point in time. The mechanism of the induction of cell death by the wild type p53 adenovirus appears to be by the induction of apoptosis by our DNA fragmentation experiments. We are presently collaborating with Dr.

McKay of this institution with electron microscopy in order to determine whether the pathoneumonic findings of apoptosis are being induced both in vivo as well as in vitro equally DNA and leveling experiments are also being performed. Importantly transduced nonmalignant cells did not exhibit the same effect as we saw as the transformed squamous cell cancers of the head and neck. This is important number 1 because there is bystander transduction that we have shown in ours as well as other investigators have shown. This transduction is involved with a replication defective virus vector and therefore permanent integration is not foreseen has a problem as compared to other potential vectors for example, retroviruses, where permanent integration in nontransformed cells may be a significant potential dilemma.

I'd like to acknowledge several people and you never can thank enough people. I have clinical and scientific mentors galore in this institution. I have a department chairman named Helmut Gefford who is immensely supportive of all of our research efforts. He is immensely supportive both clinically and from a research standpoint and none of this could be accomplished without his support. Our laboratory works tirelessly. I don't like Dr. Gefford talking about how hard I work without acknowledging all these other people. Dr. Liu, our research associate and instructor within our laboratory is here night and day and on weekends. Dorothy Taylor our laboratory coordinator has done the majority of our drug curve experiments. Xiu-Wei as well as Francois Janot – Dr. Janot was a visiting scientist here from the Gustav-Russi Institute and assisted us in these studies. I have had too numerous of scientific mentors to bring up at this time. Dr. Roth has been immensely supportive of these efforts and has generously contributed to these initial experiments and also contributed the virus that both me and Dr. Zhang have produced. Dr. Nicolson and this entire department of tumor biology have been supportive of me since day one. Doors are always open in this institution and the cross-talk that occurs between the clinician and the scientist makes this institution unique, unlike any other I think that's available. And it's an honor to be able to practice both medicine and do scientific research in this facility.

The University of Texas
M. D. ANDERSON
CANCER CENTER

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MEMORANDUM

DATE: *

TO: Gary L. Clayman, DDS, M.D.
Department of Head and Neck Surgery

FROM: Myriam Brena
Secretary, Surveillance Committee (IRB)
Office of the Vice President for Research

SUBJECT: Administrative Approval of Protocol HNS 94-001, entitled "Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"

Official Approval Date: *

Dr. Aman U. Buzdar, Surveillance Committee Chairman, reviewed and administratively approved the above named and numbered protocol. Approval needs ratification at the next IRB meeting which will be held *

It was noted that the protocol and informed consent document are satisfactory and in compliance with federal and institutional guidelines.

Patients may not be entered on this protocol until it has been officially activated by the Office of Protocol Research.

In keeping with the requirements of the Department of Health and Human Services and the Food and Drug Administration, this clinical study must be reviewed twelve months from the date of approval. If the study is terminated or completed during the next twelve months, the Surveillance Committee should be so advised. You are responsible for promptly reporting to the Surveillance Committee:

a) any severe adverse effects;
b) any death while patient is on study;
c) any unanticipated problems involving risks to subjects or others;
d) any proposed changes in the research activity (changes may not be initiated without Surveillance Committee review and approval, except where necessary to eliminate apparent immediate hazards to the subjects).

c: Michael J. Keating, M.D.

The University of Texas
M. D. ANDERSON
CANCER CENTER

M E M O R A N D U M

DATE: *

TO: Dr. Michael J. Keating
Office of Protocol Research

FROM: Myriam Brena
Secretary, Surveillance Committee (IRB)
Office of the Vice President for Research

SUBJECT: Administrative Approval of Protocol HNS 94-001, entitled "Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"

Dr. Buzdar administratively approved the subject protocol and the pertaining memo is attached. However, this protocol cannot be activated until the Surveillance Committee reviews and approves it.

Thank you for your attention to this matter.

c: Gary Clayman, DDS, M.D.

THE UNIVERSITY OF TEXAS
M. D. ANDERSON
CANCER CENTER

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M E M O R A N D U M

TO: Dr. Gary L. Clayman
Department of Head and Neck Surgery

FROM: Dr. Michael Tainsky
Vice Chairperson, Institutional Biosafety Committee

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SUBJECT: Microbial Document dated
Agent: recombinant, adenovirus type 5, CMV wildtype p53 (AdCMV p53)

The Institutional Biosafety Committee has approved the above document.

Thank you.

MT:blr
cc: Dr. Jeffrey Tarrand

THE UNIVERSITY OF TEXAS
M. D. ANDERSON
CANCER CENTER

*

M E M O R A N D U M

TO: Dr. Gary L. Clayman
Department of Head and Neck Surgery

FROM: Michael Tainsky, Ph.D. M Tainsky
Vice Chairperson, Institutional Biosafety Committee

SUBJECT: Approval of rDNA Registration Dated *
Entitled: Modification of Tumor Suppressive Gene Expression in Head and Neck Squamous Cell Carcinomas (HNSCC) with an Adenovirus Vector Expressing Wild-Type P53

The Biosafety Committee has approved the above document.

The Biosafety Committee has the responsibility of insuring compliance with regulatory agencies, such as the Recombinant DNA Advisory Committee (RAC). Before submission to RAC or any other agency, human subjects protocols and subsequent revisions pertaining to the use of recombinant DNA must receive full Biosafety Committee approval. After Surveillance Committee approval, please provide the Biosafety Committee secretary with eight copies of the human subject protocol and all correspondence received from the Surveillance Committee, RAC or any other agency.

Thank you.

MT:blr

cc:Dr. M. Frazier
Dr. R. Legerski

THE UNIVERSITY OF TEXAS
M. D. ANDERSON
CANCER CENTER

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M E M O R A N D U M

TO: Dr. Gary L. Clayman
Department of Head and Neck Surgery

FROM: Dr. Michael Tainsky
Vice Chairperson, Institutional Biosafety Committee

SUBJECT: Microbial Document dated *
Agent: recombinant, adenovirus type 5, CMV wildtype p53 (AdCMV p53)

The Institutional Biosafety Committee has approved the above document.

Thank you.

MT:blr
cc: Dr. Jeffrey Tarrand

#01

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Protocol Title: Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wild-type p53

1.

Participant's Name _____ I.D. Number _____

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so that you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other consents you may have signed in other protocols.

DESCRIPTION OF RESEARCH

2. PURPOSE OF THE STUDY: Some cancers that occur in the head and neck area may be due to a defect in a gene called p53. The purpose of this clinical research study will be to see whether a normal copy of the p53 gene can be placed inside the patient's cancer cells and cause the cancer to grow more slowly or to stop growing.

3. DESCRIPTION OF RESEARCH: To introduce the normal p53 gene into tumor cells utilizing a defective virus of the adenovirus type which is similar to other viruses that cause the common cold. This defective virus is called a "vector". These tumors will be injected directly with the adenovirus three times a week for two weeks. After two weeks of rest from receiving treatment, tumors will then be injected again with the virus three times a week for two consecutive weeks. If the tumor can be removed in its entirety, surgery will be performed for removal of tumor that is considered completely removable by the patient's physician. Surgery must be performed within four days after completion of the last injection of virus. At the time of surgery, additional virus is placed into the area where the tumor has been removed. Tubing, which is ordinarily placed in surgical areas, is used to allow for drainage of fluids from the surgery area. Three days after the tumor has been removed, virus will be placed into the tubing and allowed to enter the surgical site once again. If the patient has undergone surgery, this will be the last treatment with adenovirus.

If the cancer cannot be removed by the physician, this tumor will be injected directly

three times a week for two consecutive weeks. After two weeks of rest from treatment, courses of three injections weekly for two consecutive weeks are repeated on a monthly basis. Injections of adenovirus are continued if tumor continues to shrink. If there is absence of tumor shrinkage, evidence of tumor growth, or adverse reactions to the adenovirus injections, treatment will be terminated.

The injections into the tumor are delivered with a skinny needle. If the tumor is greater than approximately two inches in size, approximately two teaspoons of virus will be injected into the tumor. Smaller tumors, less than two inches, will be injected with less than one teaspoon of virus. Multiple injections of virus into the tumors will be required with these injections being spaced apart by approximately one-half inch each. Numbing medication may be placed on the overlying skin to decrease discomfort from injections.

Patients with tumors of the throat or the voice box may also receive the treatment. It may be necessary to remove a part of the tumor surgically or with a laser before the treatment with p53 is given. Other patients may require that a tracheostomy is first performed. A tracheostomy is a surgical operation to make an opening into the windpipe. Certain routine diagnostic studies will be performed before entry into this trial. These involve local examination of the tumor by inserting an instrument with a light into the throat.

If previous specimens are insufficient for laboratory studies related to this research, additional biopsies will be needed. The treatment will be repeated monthly as long as there is evidence that the tumor is not growing.

The experimental treatment and costs related to the patient's participation in this research and which include clinical examinations, biopsies, and other forms of testing will be provided free to the patient. A maximum of 42 patients will be entered in this study. Twenty one patients may have injections of adenovirus with surgery and twenty one patients may have injections of adenovirus without surgery.

The patient's course will be followed indefinitely. Dr. Clayman's office should be notified if an address change is made.

PERMISSION FOR AUTOPSY: In case of death, the family of the patient will be asked for permission to perform an autopsy.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

Two small additional biopsies will be required in addition to the initial biopsy. Risks from biopsies include coughing up blood which is usually slight. Severe hemorrhage which requires emergency treatment is rare. Biopsy of neck masses may also be associated with a slight risk of bleeding or infection. This clinical research study may involve unforeseeable risks to the participant.

Possibility of Causing a New Cancer. It is possible that the research could cause cancer in normal cells although this risk is believed to be small when the injected virus has been properly safety tested prior to its use. The adenovirus vector has only been used, to date, on the lining of the breathing tube in over twenty patients with no ill effects noted.

4a. This clinical procedure may involve unforeseeable risks to the unborn children, therefore, the participants should practice adequate methods of birth control throughout the period of their involvement in the clinical study if they are sexually active. To help prevent injury to children, the female participants should refrain from breast feeding during participation in the clinical research study.

5. POTENTIAL BENEFITS:

This treatment may result in shrinkage of the tumor, which may decrease cancer associated symptoms or may prolong life.

6. ALTERNATE PROCEDURES OR TREATMENTS:

Chemotherapy or other experimental drugs may be an alternative for some individuals. These treatments cause shrinkage of cancer in a number of patients. Another option is to only control the symptoms of disease and not take chemotherapy treatment.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the procedure involved and the investigator has been willing to reply to my inquiries. This procedure will be administered under the above numbered, title, and described clinical research protocol at this institution. I hereby authorize Dr. the attending physician/investigator and designated associates, to administer this procedure.

8. I have been told and understand that my participation in this clinical research study is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or

effects of my decision with my physician.

In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

I will be informed of any new findings developed during the course of this clinical research study which might be relevant to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration, Microbiological Associates, Magenta Corporation (manufacturers of the virus), and National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that, should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available for treatment at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for such injury.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost.

Costs related to my medical care will be covered by the Introgen Sponsored Research Agreement. Clarification of specific cost issues may be addressed in section 3 of this informed consent. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, devices, new drugs, or possible patentable procedures, in which event I understand that I cannot expect to receive any compensation or benefits from the subsequent use of information acquired and developed through my participation in this research project.

13. I understand that refraining from breast feeding and practicing effective contraception is medically necessary and a prerequisite for my participation in this clinical research

. IRB Approved Consent
. Date of Activation *
. Signature

study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.

14. I may discuss any questions or problems during or after this study with Dr. Gary L. Clayman at [redacted] In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman or the Surveillance Committee at [redacted] I may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based upon the above, I consent to participate in the research and have received a copy of the consent form.

DATE

SIGNATURE OF PARTICIPANT

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

I have discussed this clinical research study with the Participant and/or his or her authorized representative using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe the participant understood this explanation.

PHYSICIAN/INVESTIGATOR

- IRB Approved Consent
- Date of Activation *
- Signature

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Protocol Title: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53*

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PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title) (Two lines not to exceed 75 characters per line)

Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53

Study Chairman: Gary L. Clayman, D.D.S., M.D., Department of Head and Neck Surgery

Patient Eligibility: (Twenty lines not to exceed 75 characters per line)

1. Patients must have histologic proof of squamous cell carcinoma of the head and neck. Patients must be either unable to receive conventional treatment (e.g. the patient received radiation therapy with or without surgery) or have failed conventional treatment. Those patients with extensive local or regional disease that have persisted or recurred following radiation therapy (with or without chemotherapy or surgery) and have clinically resectable, but likely non-curable (<10% disease free survival) are also eligible. Patients need not have received a trial of chemotherapy prior to entering this protocol. All eligible patients will be discussed at the Head and Neck Surgery Multidisciplinary Treatment Planning Conference prior to protocol enlistment.
2. Patients must have clinical evidence of advanced local and/or regional cancer which is unresectable or for which no meaningful resection with surgical margins will be obtainable.
3. All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).
4. All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.
5. Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative. Patients with upper respiratory infections will not be treated until the infection resolves.
6. Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of >2,000/mm^3 and platelet count of 100,000/mm^3), adequate liver function (bilirubin ≤ 1.5 mg/dl), and adequate renal function (creatinine <1.5 mg/dl).

Treatment Plan: (Include dose adjustment) (Twenty lines not to exceed 75 characters per line)

1. The study will be an open-label upward dose ranging study for adenovirus-p53 vector (Ad5CMV-p53) in two patient groups. The two groups of patients will consist of a) resectable and b) non-resectable recurrent disease. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive on intratumor injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU).
2. Dose Escalation: The adenovirus dose will increase in one $\log_{10}$ increments for each group until 10^9 PFUs are reached. Patients entered at a given dose level will not be eligible for dose escalation. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients in each group (resectable and non-resectable).
3. All patients shall be registered with the Data Management Office
4. Patients with local-regional tumors will have injection of a total dose of 10 ml for tumors ≥ 4 cm in diameter or 3 ml for tumor <4 cm in diameter of the adenovirus preparation with the appropriate number of viral particles at multiple sites percutaneously or transorally. The treatment will be repeated three times weekly for two weeks. Dose escalation may proceed after a minimum two week follow-up of the last patient entered into the previous dose level. Treatment will continue on a monthly basis as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.
5. Those patients with surgically resectable disease will be treated by tumoral injection of adenovirus preparation as described above. The treatment will be repeated for two consecutive courses. Within four days of completion of the second course, the patients will be eligible to proceed with surgical resection. Prior to surgical closure, 10 ml of adenovirus preparation will be administered into the surgical defect (operative bed) and allowed to remain in contact for 60 minutes. The wounds are then closed and drains placed.

Patient Evaluation: (Twenty lines not to exceed 75 characters per line)

1. A complete history and physical to include performance status, recent weight loss, usual weight and concurrent non-malignant disease and its therapy, and all prior anticancer treatments must be recorded. History and physical will be performed prior to each course.
2. Laboratory studies shall include quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, HIV analysis, urinalysis, chest x-ray, pre and post-treatment.
3. All relevant information regarding viral dosage, tumor response, laboratory examinations, and treatment-related toxicities must be recorded before each treatment is given.
4. Core biopsies or Incisional biopsies will be obtained of accessible local and or regional tumor. Tumor specimens will be collected 72 hrs. after the last injection of the adenovirus during the first treatment cycle.
5. Biopsies will be analyzed for incorporation of the transduced gene into the host genomic DNA and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction and by in situ hybridization.
6. All patients will be evaluable for response and toxicity following one course of therapy.
7. A blood sample will be collected three times at one-half hour intervals following injection of the adenovirus. These samples will provide leukocytes to analyze for uptake of adenovirus DNA. Serum will be tested for antibodies to adenovirus proteins. Patients will be tested monthly during treatment, monthly for the first three months following completion of treatment, every three months for the remainder of the year following completion of treatment, and then at least yearly thereafter.
8. Normal tissue samples will be collected during the follow-up visits and endoscopies. These will include samples of non-malignant mucosa, leukocytes, and germ cells, if possible. These tissues will be analyzed for incorporation of the adenovirus.
9. A staging CT scan of the head and neck to evaluate local and regional disease will be obtained on an every three month basis during treatment.

Miscellaneous Information: (Include any other information that you feel is pertinent to the study)
(Three lines not to exceed 75 characters per line)

Statistical Considerations: (Twelve lines not to exceed 75 characters per line)

Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitations imposed by production of the adenovirus). A maximum of 21 patients will be entered into each study group, for a total of 42 patients for the entire study.

Objectives: (Twelve lines not to exceed 75 characters per line)

1. To determine the maximum tolerated dose of the wild-type p53 adenovirus vector in patients with refractory NSCC.
2. To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.
3. To document observed antitumor activity of this treatment approach.

Clinical Protocol: Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"

1.0 OBJECTIVES

1.1 To determine the maximum tolerated dose of the wild-type p53 adenovirus vector in patients with refractory HNSCC.

1.2 To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.

1.3 To document observed antitumor activity of this treatment approach.

2.0 BACKGROUND AND RATIONALE

2.1 Molecular events in HNSCC

Head and Neck Squamous Cell Carcinoma accounts for nearly 45,000 thousand new cancers per year in the United States and in several parts of the world is one of the most frequent cancers. Nevertheless, mortality remains at nearly 55% and has not significantly changed since contemporary radiation therapy was implemented over thirty years ago. Patients with HNSCC are afflicted with a disease process which may have profound effects on speech, swallowing, cosmesis, and frequently cause social isolation. Unfortunately, all current treatment modalities, including radiation therapy, surgery, and chemotherapy, continue to have limited effectiveness in patients with advanced disease. Local/regional control remains a major issue in this patient population with only approximately 10% of patients with advanced disease succumbing to distant disease alone. The rational development of new therapies for HNSCC will depend on an understanding of the biology of this cancer at the molecular level. Molecular analysis has identified critical molecular events leading to HNSCC development and progression. The goal of this research is to directly modify the cancer cell to express large quantities of exogenously introduced a normal tumor suppressor gene product that suppresses the characteristics of the malignant phenotype.

The purpose of this protocol is to investigate molecular mechanisms that may influence the growth and progression of HNSCC; our goal is development of therapeutic agents specifically targeted at the molecular level. Approximately 80-85% of head and neck cancers are squamous cell carcinomas. Much of the current data on the molecular progression model of HNSCC has shown that multiple molecular events occur in the carcinogenic cascade. Although molecular studies have shown p53, int-2, Rb, Prad-1, and c-erb B2/Neu may all be involved in HNSCC, loss of heterozygosity microsatellite analysis has shown 3p, 9p, 14q, and 17p are frequent sites of loss (>40%) in adjacent premalignant areas.[1] The control and survival rates of patients with advanced head and neck cancers are low despite aggressive local regional therapy. Although dramatic responses have been observed with chemotherapy, its impact upon survival is minimal. Established biologic predictors of chemo or radiation therapy response have remained elusive. HNSCC also may serve as a model for other carcinogen-induced malignancies. The approaches and observations developed in this study may be applicable to other types of epithelial cancers.

Abundant evidence has accumulated that the process of malignant transformation is mediated by a genetic paradigm[2]. The major lesions detected in cancer cells occur in dominant oncogenes and tumor suppressor genes. Dominant oncogenes have alterations in a class of genes called proto-oncogenes, which participate in critical normal cell functions, including signal transduction and transcription. Primary modifications in the dominant oncogenes that confer the ability to transform include point mutations, translocations, rearrangements, and amplification. Tumor suppressor genes appear to require homozygous loss of function, by mutation, deletion, or a combination of these for transformation to occur. Some tumor suppressor genes appear to play a role in the governance of proliferation by regulation of transcription. It is possible that modification of the expression of

dominant tumor suppressor genes may influence certain characteristics of cells that contribute to the malignant phenotype.

Despite increasing knowledge of the mechanisms involved in oncogene-mediated transformation, little progress has occurred in developing therapeutic strategies that specifically target oncogenes and their products. Initially, research in this area was focused on dominant oncogenes, as these were the first to be characterized. DNA-mediated gene transfer studies showed acquisition of the malignant phenotype by normal cells following the transfer of DNA from malignant human tumors. Activated oncogenes of the *ras* family were identified by this technique with transfection of human DNA into mouse NIH 3T3 cells. More recently a class of tumor suppressor genes have been identified. Mutation or deletion of both copies of a tumor suppressor gene is required to eliminate its function and cause the cell to acquire characteristics of the malignant phenotype.

Tumor Suppressor Gene Mutations in Head and Neck Squamous Cell Carcinoma

The *p53* gene is the most frequently mutated gene yet identified in human cancers. It is mutated in over 50% of human HNSCC[3]. The *p53* gene encodes a 375-amino-acid phosphoprotein that can form complexes with viral proteins such as large-T antigen and E1B[4]. Missense mutations are common for the *p53* gene and are essential for the transforming ability of the oncogene. The wildtype *p53* gene may directly suppress uncontrolled cell growth or indirectly activate genes that suppress this growth. Thus, absence or inactivation of wildtype *p53* may contribute to transformation. However; some studies indicate that the presence of mutant *p53* may be necessary for full expression of the transforming potential of the gene. Mutations of *p53* are common in a wide spectrum of tumors[5-8]; they occur in both HNSCC cell lines and fresh tumors[9]. Additionally, they occur in second primary cancers that may arise in over 20% of head and neck cancer patients.[10]

An option for specific targeting of tumor suppressor genes is replacement of a deleted or mutated tumor suppressor gene. Progress in the understanding of the critical genes involved in tumor development and in technology for altering gene expression logically led to our studies of techniques for achieving these options.

Our working hypothesis is that overexpression of a wildtype *p53* tumor suppressor gene in the cancer cell can potentially reverse critical features of the malignant phenotype of that cell. This finding has important therapeutic implications. Cancer cells have multiple genetic alterations. Therapy directed toward oncogenes will be practical only if therapeutic effects occur with targeting of one or two genes. It is unlikely that any therapy targeting oncogenes or their products will be absolutely specific for cancer cells. If other genes can compensate for loss of normal function by a specific oncogene mediated by an antisense construct, the harmful effects of the therapy will be reduced. Studies from Roth et al. indicate that reversal of a single genetic alteration has profound effects on the growth and tumorigenicity of carcinoma cells[11]. Additional support for this concept comes from a recent study by Soriano and co-workers[12] in which transgenic mice were created that lacked a functional *c-src* proto-oncogene. The resulting developmental defect in the mice was osteopetrosis. The ubiquity of *c-src*, its high degree of conservation among species, and its role in mitosis suggest that inactivation would be lethal, but this was not the case; viable mice were recovered. A possible explanation is that other closely related nonreceptor tyrosine kinases such as *yes* and *fyn* can compensate for loss of *c-src*. Introduction of a single copy of a wildtype tumor suppressor gene into normal cells would be unlikely to have adverse effects if it occurred during therapy directed at replacing inactivated tumor suppressor genes in cancer cells. To support this, work in our laboratory has investigated the effect of exogenous wild-type *p53* transduced in non-malignant fibroblasts and found that no alteration in cell growth or morphology occurs as compared to tumor cells that undergo cell cycle arrest, inhibition of tumorigenicity and apoptosis. (Clayman, unpublished data Appendix D. Figure 6.)

Wild-type *p53* appears dominant over its mutant gene and will select against proliferation when transfected into cells with the mutant gene[11,13]. Our experiments have shown that expression of the transfected wt*p53* does not affect the growth of non-malignant cells with endogenous wt*p53*. Thus, such constructs might be taken up by normal cells without adverse effects. This protocol will study

local or regional delivery of wt*p53* to HNSCC patients with unresectable regionally metastatic squamous cell carcinomas, unresectable local cancers, or advanced local and/or regional HNSCC that have failed prior treatment which included radiation therapy but remains locally and regionally resectable. The efficiency of delivery and gene expression will be evaluated both in head and neck cancer cells and in nor mal cells in vivo. This is of importance for the design of constructs that may be useful therapeutically. The effects of these constructs on clinical progression of the cancer will also be studied.

These approaches may lead to cancer therapy based on direct alteration of gene expression in cancer cells. Current therapy relies on attempts to kill or remove the last cancer cell. However, tumor cell dormancy is an established phenomenon making effective killing highly unlikely. Although inhibition of expression of some oncogenes may be lethal to the cancer cell, in some cases cell replication will slow or cease, thus rendering these cancers clinically dormant. Nevertheless, the chosen delivery method of adenovirus vectors addresses this dormancy issue since transduction by the vector is cell cycle independent. Even if absolute specificity is not achieved, single oncogenes or tumor suppressor genes may still be important targets, because it is likely that adverse effects to normal cells will be minimal as well as transient with the transient adenovirus vector chosen.

Programmed cell death, also known as apoptosis, shows a characteristic pattern of DNA fragmentation resulting from cleavage of nuclear DNA and is considered to be a selective process of physiologic cell deletion. It has recently been reported that the wt*p53* gene is involved in mediating programmed cell death of some types of tumor cells[14-16]. Our studies have shown that independent of the endogenous p53 status (homozygous mutated or wild-type), HNSCC cells transduced with recombinant adenovirus-mediated wt*p53* gene exhibit histologic alterations both by light field and electron microscopy that are consistent with apoptosis. Similarly, in vitro infection with the same vector induces specific DNA fragmentation consistent with this mechanistic hypothesis. Nevertheless, this is in contrast to normal cells with wildtype p53 which are unaffected by wildtype p53. Direct injection of adenovirus *p53* into subcutaneously established and microscopic HNSCC tumors in nu/nu mice induces apoptosis and dose response destruction and inhibition of tumor development and growth.(Clayman, unpublished data, Appendix D) These changes occur independent of the HNSCC tumor cell line endogenous p53 status (wildtype or mutated). These results support the use of this strategy in a clinical trial.

2.2 Natural history of locally unresectable HNSCC and M.D. Anderson Patient Patterns

Patients with HNSCC die of their cancer in approximately 55% of cases and failure of therapy at the primary or regional tumor site is a significant problem[18,19]. Of the 45,000 patients newly diagnosed with head and neck cancer in 1991, nearly half underwent surgical resection with or without radiation therapy. Local and or regional recurrence as the first site of failure will occur in approximately 22,000 of all of those patients independent of the treatment modality chosen (surgery, radiation therapy, chemotherapy or combinations thereof). Thus, nearly 22,000 patients per year could benefit from improved local-regional therapy. Patients with local/ regionally unresectable HNSCC, that has failed radiation therapy, have a median survival of approximately 6 months and no known systemic chemotherapy has shown significant survival benefit among these patients. The Department of Head and Neck Surgery at the University of Texas M. D. Anderson Cancer Center has extensive experience in the treatment of patients afflicted with squamous carcinoma of the upper aerodigestive tract. Over 1200 patients with head and neck cancer are seen yearly and over 600 of these patients undergo resections. From September 1992 to August 1993, 469 patients with local/regional squamous cell of the upper aerodigestive tract (no evidence of distant metastasis) were referred to the Department of Head and Neck Surgery at M. D. Anderson Cancer Center; 113 had prior radiation therapy (52 radiation therapy only, 40 surgery and radiation therapy, 13 chemotherapy and radiation therapy, and 8 chemotherapy + radiation therapy + surgery). Of these 113, 84 had local/regional squamous cell carcinoma without distant metastasis.

2.2.1 Measure of disease activity

The goal of this therapy is to halt or reverse the manifestations of the disease. The efficacy of

therapy in this group of patients will be measured by determining length of patient survival and reduction in measurable tumor mass. There is no curative therapy for this stage of disease and thus the outcome is predictable enough to allow for an assessment of the results of gene therapy. The measurements that will be used are described in Section 7.0.

2.2.2 Anticipated effect of protocol treatment

It is anticipated that the administration of the adenovirus wildtype *p53* will decrease the rate of proliferation of these cells and induce apoptosis of infected malignant cells. This would reduce the growth rate or cause regression of primary and/or nodal disease and therefore relieve symptoms and potentially prolong the patient's survival.

2.2.3 Alternative therapies

Patients with unresectable squamous cell carcinoma of the head and neck that have failed or are unable to receive external beam radiotherapy will be considered for this protocol. Existing therapies for this condition offers only the potential for short-term palliation. Most patients have recurred despite external beam radiotherapy.Patients receiving this treatment have a median survival of approximately 6 months. Patients failing brachytherapy would also be eligible to receive gene therapy. In those patients that may still have potentially resectable tumor, which has failed radiation therapy (alone or in combination with prior surgery or chemotherapy), in which tumor can be surgically excised following adenovirus *p53* gene therapy and then additional gene therapy can be delivered to the surgical bed after the tumor volume has been drastically reduced to microscopic disease will also be eligible. These patients, although technically resectable have the same prognosis as unresectable patients. Patients with unresectable local-regional tumors who have failed surgery or radiation therapy have a poor prognosis. Chemotherapy is only palliative and the median survival remains less than 6 months. The administration of the adenovirus constructs would not preclude the patient from receiving other palliative therapy if the tumor progresses.

2.3 Structure and characteristics of the biological system

2.3.1 Restoration of expression of wt*p53* gene product

2.3.1.1 Preliminary studies with plasmid DNA

The *p53* gene is the most commonly altered gene yet described in human cancers. To study this gene, a cell culture model system of cell lines varying in *p53* expression was established. The H322a adenocarcinoma cell line expresses the mutant *p53* protein as shown by the presence of high levels of endogenous *p53* mRNA and phosphorylated protein. We showed that the H322a cell line has a G:T transversion at codon 248 (Arg to Leu) with absence of the wildtype allele. The H322a cell line has a homozygous *p53* deletion. The H460a and H226b cell lines are homozygous for the wildtype *p53*. Expression vectors for sense (S-*p53*) and antisense *p53* (AS-*p53*) cDNA with a ß-actin promoter were constructed to study the effect of wt*p53* expressed in lung cancer cells with mutant or deleted *p53* and the effects of reducing wildtype and mutant *p53* expression[11].

Stable transfectants of *p53* mutant cells (H322a) or deleted *p53* (H358) expressing S-*p53* could not be rescued. Failure to isolate colonies expressing sense *p53* RNA in cells with homozygous mutant or deleted alleles shows that wt*p53* can suppress in vitro cancer cell growth in cells expressing a mutant *p53* or having a homozygous *p53* deletion.

In general, transfection with antisense *p53* (AS-*p53*) reduced colony formation (10-fold) by cells with endogenous mutant *p53*. This indicates that expression of mutant *p53* contributes to the transformed phenotype. As expected, cells with wt*p53*

(H226b) showed increased tumorigenicity when transfected with AS-*p53*. The H226b cells expressing AS-*p53* grow significantly more rapidly in nu/nu mice than the cells transfected with the control plasmid. This indicates that elimination of the wt*p53* gene product enhances features of the malignant phenotype.

These studies showed that wt*p53* is dominant and can suppress the malignant phenotype in cells with mutant or deleted *p53*. The presence of the mutant *p53* confers transforming potential to the gene product, which can be suppressed by AS-*p53*. Thus, in cancer cells both the absence of wt*p53* and the presence of certain *p53* mutations may enhance the malignant phenotype.

2.3.1.2 Generation of recombinant p53 adenovirus.

The p53 expression cassette (Figure 1, Appendix D), which contains human cytomegalovirus (CMV) promoter[21], wild-type p53 cDNA, and SV40 early polyadenylation signal, was inserted between the Xba I and Cla I sites of pXCJL.1, a plasmid kindly provided by Dr. Frank L. Graham of McMaster University, Hamilton, Ontario, Canada. The p53 shuttle vector (pEC53) and the recombinant plasmid pJM17[22] were cotransfected into 293 cells[23] by liposome-mediated transfection with DOTAP (Boehringer Mannheim Corp., Indianapolis, IN)[24]. The transfected cells were maintained in medium until the onset of the cytopathic effect. Identification of newly generated p53 recombinant adenoviruses (Ad5CMV-p53) with PCR analysis of the DNA samples prepared from the cell culture supernatants was described elsewhere[24]. The wild-type sequence of the p53 cDNA in the Ad5CMV-p53 virus was confirmed by dideoxy DNA sequencing on the CsCl-gradient-purified viral DNA. The control virus Ad5/RSV/GL2, generated in a similar manner, has a structure similar to that of Ad5CMV-p53 except a Rous sarcoma viral promoter and luciferase cDNA were used in its expression cassette. The recombinant adenovirus that carries a *E. coli* b-galactosidase gene (LacZ), Ad5CMV-LacZ, also has a structure similar to that of Ad5CMV-p53, and was kindly provided by Dr. Frank L. Graham. Structural analysis of the vectors is shown in Fig. 2, Appendix D.

2.3.1.3 Viral stock, titer, and infection. Individual clones of the Ad5CMV-p53, Ad5/RSV/GL2, and Ad5CMV-LacZ viruses were obtained by plaque-purification according to the method of Graham and Prevec[25]. Single viral clones were propagated in 293 cells. The culture medium of the 293 cells showing the completed cytopathic effect was collected and centrifuged at 1000 x *g* for 10 min. The pooled supernatants were aliquoted and stored at -20°C as viral stocks. The viral titers were determined by plaque assays[25]. Infections of the cell lines were carried out by addition of the viral solutions (0.5 ml per 60-mm dish) to cell monolayers and incubation at room temperature for 30 min with brief agitation every 5 min. This was followed by the addition of culture medium and the return of the infected cells to the 37°C incubator.

2.3.1.4 Preclinical studies

Expression of exogenous p53 protein in HNSCC cells. To obtain a high level expression of p53, the human CMV promoter[21] was used to drive the p53 cDNA carried by Ad5CMV-p53. As shown by immunostaining and Western blot in Figures 3 and 4 (Appendix D), a high level of expression of exogenous p53 was achieved in the HNSCC cell lines Tu138 and Tu177 that were infected by Ad5CMV-p53 at an MOI of 50 PFU/cell. When Tu138 or Tu177 cells were infected at the same MOI, the level of expression of the exogenous wild-type *p53* gene was at least four times higher than that of the endogenous mutated protein in Tu138 and 14 times higher than that of the endogenous wild-type protein in MDA 1986 cells (data not shown). The time course of the expression of the exogenous *p53* after a single infection of 10 PFU/cell was studied in H358 cells. The protein expression peaked at post-infection day 3, sharply decreased after day 5, and lasted for at least 15 days (Fig. 5, Appendix D).

PCR analysis on the DNA samples prepared from the Ad5CMV-p53-treated H358 cells failed to detect the viral DNA after post-infection day 15 (data not shown). The decrease in expression of the exogenous *p53* probably resulted from the cellular attenuation on the CMV promoter or degradation of the viral DNA in the treated cell population[26]. This is a critical point with respect to safety of the vector. Transient *p53* expression is sufficient for mediating apoptosis. However, normal cells taking up the vector will express the exogenous *p53* for only a short time.

Effect of exogenous p53 on HNSCC cell growth. Five human HNSCC cell lines were chosen for this study: cell line Tu138 and Tu177, which both have homozygous mutations of the *p53* gene, and cell lines MDA 686, 886 and 1986 which have wild-type *p53* genes. Additionally, fibroblasts derived from stromal tissue culture outgrowth of patients afflicted with head and neck cancer were also assessed. The cells were treated with Ad5CMV-p53 and dl312 (replication defective adenovirus control) at 50 PFU/cell. Triplicate sets of the viral-infected and mock-infected cells were counted every day for 6 days. Growth rates of the Ad5CMV-p53-infected HNSCC cells were significantly inhibited compared to that of the mock-infected cells and dl312 infected cells. (Fig. 6., Appendix D). Unlike other epithelial cancer cell lines previously tested, within 72 hours post-infection, no viable cell colonies can be obtained in reculturing transduced cell culture dishes. Twenty-four hours after infection, an apparent morphologic change occurred with portions of the cell population rounding up and their outer membranes forming blebs. Cells infected with dl312, (replication-defective adenovirus), demonstrated normal growth characteristics with no histomorphologic abnormalities. Ad5CMV-p53 had no effect on cell growth or morphology in non-malignant fibroblasts derived from cancer patients. (Fig. 6, Appendix D)

Inhibition of tumorigenicity mediated by Ad5CMV-p53. To examine whether the Ad5CMV-p53 virus can inhibit tumorigenicity of human HNSCC cells, the effect of AdCMV-p53 on established subcutaneous tumor nodules was determined in nude mice in a defined pathogen-free environment. Briefly, following induction of acepromazine anesthesia, three separate s.c. flaps were elevated on each animal and 5×10^6 cells in 150µl nude mice were injected s.c. After four days, palpable tumors were noted in each pocket site ($6mm^3$ or greater in size) and animals were reanesthetized, and the flaps were re-elevated for the delivery of 100µl of a) Ad5CMVp53 (10^8 P.F.U.) in the right anterior flap; b) replication defective adenovirus, dl312, (10^8 P.F.U.) in the right posterior flap and c) transport medium alone in the left posterior flap. Animals were observed daily and sacrificed on day 20 following experimental interventions.

Seven animals were tested for each cell line. One animal in the Tu-177 group died following the second flap surgery and delivery of the therapeutic interventions, presumably due to profound anesthesia and subsequent mutilation by cage mates. Necropsy revealed no evidence of metastasis or systemic effects. Figure 7, Appendix D shows representative Tu-138 (left) and Tu-177 recipients (right). Sizable tumors are apparent on both posterior flaps of the animals (i.e., the sites that did not receive Ad5CMV-p53). The lack of tumor progression is significant in the right anterior flaps of the animals, which received Ad5CMV-p53 in both cell lines tested. That Tu-177 cells have a slower growth rate has previously been established in these animals (unpublished data). Two animals in the Tu-177 group had complete clinical and pathologic regression of their established subcutaneous tumor nodule. Two animals in the Tu-138 group were killed early because they were experiencing rapid growth and ulceration of the control tumor sites. All surgical sites had developed lesions of at least 6 mm^3 before intervention. The tumor volumes on necropsy are shown in Table 1.

Table 1 Effect of Ad5CMV-p53 on Established tumor growth in nude mice[a]

Treatment	Mean volume [$mm^3 \pm$ SEM] Tu-138 (7)	Tu-177 (6)
Ad5CMV-p53	22.3 ± 14	13 ± 18
Ad5(dl312)	803 ± 300	533 ± 148
Medium	1297 ± 511	421 ± 143
Significance	*p* value	*p* value
p53[b] : dl312	0.03	0.02
p53 : Medium	0.04	0.03

[a]The cells were injected subcutaneously at 5 x 10^6 cells/flap. Tumor sizes were determined at day 20 after treatment. Numbers in parentheses represent the number of animals evaluated.
[b]Ad5CMVp53 is abbreviated as p53; dl312 is an abbreviation for Ad5(dl312). Statistical anallysis by Friedman's two-way anallysis of variance.

The efficacy of Ad5CMV-p53 in inhibiting tumorigenicity was further evaluated in the mouse model of microscopic residual disease in HNSCC. Representative HNSCC cell lines of homozygous mutations, as well as wild-type *p53* cell lines were used. In head and neck cancer, as well as several other solid malignancies, direct gene transfer to microscopic residual carcinoma may not be so technically difficult. When the primary source of tumor is removed, the tumor base is readily available for molecular therapy as well as the most likely pathway of lymphatic spread when a neck lymph node dissection is performed. To investigate these issues, we designed our experiments to determine if in vivo adenovirus *p53* mediated gene transfer would effect the establishment or growth of SCCHN cells implanted into a subcutaneous flap. Athymic nude female mice were anesthetized and three subcutaneous flaps elevated and the SCCHN cell lines pipetted subcutaneously in order to prevent erroneous tumor inoculation and dispense a specified number of cells. Following 48 hours, mice were reinoculated with either adenovirus *p53*, transport medium alone, and a non-marker replication defective adenovirus dl312 or the adenovirus ß-galactosidase vector. The development of tumors was tumor cell number dependent, allotted time for implantation dependent, and dose dependent upon adenovirus *p53*. A representative viral dose response experiment is shown in figure 8, Appendix D. Dose response experiments with the marker Galactosidase gene clearly demonstrate dose-response transduction efficiency in this model (figure 8) and was also confirmed by p53 immunohistochemistry three days following infection (figure 9, Appendix D). In the presence of microscopically implanted tumor of 2.5-5.0 x 10^6 cells treated with adenovirus *p53* at 10^8 P.F.U. or greater, tumors developed in 2 of 6 animals in only one of the wild-type cell lines (886LN). All other tumor cell line exhibited total inhibition of tumor development both grossly and histologically. These experiments clearly indicate that microscopic proliferating tumor cells can be successfully infected in vivo if exposed to adenovirus *p53*. Tumor formation was evaluated at the end of a 8-week period by gross and histologic analysis of the surgical sites. The data of tumor measurements are summarized in Table II. These results indicate that Ad5CMV-p53 can prevent the formation of HNSCC in a subcutaneous model of microscopic residual disease.

Table II. Effect of Ad5CMV-p53 on Tumorigenicity in a Microscopic Residual Disease Model of HNSCC.[a]

Cell line	Treatment		
	No. mice w/ Total Mice (%)		
	Vehicle (PBS)	dl312	Ad5CMV-p53
Tu138 (homozygous mutation p53)	8/8	8/8	0/8
Tu177 (homozygous mutation p53)	8/8	8/8	0/8
686 LN (homozygous wild-type p53)	5/8	5/8	0/8
886 (homozygous wild-type p53)	6/6	6/6	2/6

[a] Mice were inoculated with 2.5 x 10^6 /flap subcutaneously. On the 2nd day post-inoculation, the mice were given either vehicle or viruses (1 x10^8 P.F.U. each in 0.1 ml) in the same flap site in a single intervention strategy. Tumor formation was evaluated at the end of a 8-week period.

In a treatment strategy to determine the role of repeat treatment of AdCMVp53 in established tumors, subcutaneously established HNSCC tumor cell lines were peritumorally infiltrated with either vehicle (PBS) or viruses (1 x10^9 P.F.U. each in 0.1 ml) three times weekly for two consecutive weeks. Tumor burdens exceeding 1 cm in greatest dimension were utilized in these studies. A greater than 50% reduction in Tu138 tumor mass was seen in 5/5 animals treated in this study with significant reduction in size compared to control-treated sites ($p < 0.04$). (Figure 10, Appendix D) No evidence of systemic toxicity was clinically or histologically noted in whole organ necropsy studies. In additional repeat treatment studies using smaller established tumors that had reached 75mm^3, were similarly peritumorally treated with identical controls as described above. In both Tu177 and Tu138, following 7 peritumoral AdCMVp53 treatments (3 times weekly), 8 of 9 animals had complete regression of disease whereas control animals showed continued progression of tumors as well as no evidence of systemic toxicity in every animal (10 of 10).

3.0 SAFETY INFORMATION

3.1 Continued absence of replication competent infectious virus was determined from sequential infection experiments. No replicative virus was detectable by PCR analysis of DNA samples from HeLa cells treated with the frozen/thawed cell extracts from HeLa cells initially infected with Ad5CMV-p53 at 100 PFU/cell, Ad5CMV-p53 was confirmed as a replication-defective and helper-independent virus. Further confirmation of this was obtained by labeling viral supernatants with [^{3}H]Thymidine. Absence of labeling in extracted DNA showed absence of replication competent adenovirus. These studies and the following safety studies will be performed by Microbiological Associates, Inc.

3.2 Sterility will be assured by testing for aerobic and anaerobic bacteria, fungus, and mycoplasma. Other tests to be performed by Microbiological Associates, Rockville, MD include:

Transmission EM for Viruses
In Vitro Assay for Adventitious Viral Contaminants
In Vivo Assay for Adventitious Viral Contaminants
Isoenzyme & Cytogenetic Analysis
Tumorigenicity$_c$
EBV
CMV
Hepatitis
HIV Co-Cultivation
HTLV 1/2 PCR
Adeno-Associated (AAV) Hybridization
Parvovirus B-19 Hybridization Adenovirus

4.0 PATIENT ELIGIBILITY

4.1 Patients must have histologic proof of squamous cell carcinoma of the head and neck. Patients must be either unable to receive conventional treatment (e.g. the patient received radiation therapy with or without surgery) or have failed conventional treatment. Those patients with extensive local or regional disease that have persisted or recurred following radiation therapy (with or without chemotherapy or surgery) and have clinically resectable, but likely non-curable (<10% disease free survival) are also eligible. Patients need not have received a trial of chemotherapy prior to entering this protocol. All eligible patients will be discussed at the Head and Neck Surgery Multidisciplinary Treatment Planning Conference prior to protocol enlistment.

4.2 Patients must have clinical evidence of advanced local and/or regional cancer which is unresectable or for which no meaningful resection with surgical margins will be obtainable.

4.3 All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤2 (Zubrod scale, Appendix B).

4.4 All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.

4.5 Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative. Patients with upper respiratory infections will not be treated until the infection resolves.

4.6 Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of ≥2,000/mm^3 and platelet count of ≥100,000/mm^3), adequate liver function (bilirubin ≤1.5 mg/dl), and adequate renal function (creatinine ≤1.5 mg/dl).

4.7. Female patients of child-bearing potential are excluded.

5.0 TREATMENT PLAN

5.1 The study will be an open-label upward dose ranging study for adenovirus-*p53* vector (Ad5CMV-p53).

5.2 The study will be done with two groups of patients. The two groups of patients will consist of a) resectable and b) non-resectable recurrent disease (Refer to 4.1). It is not known what toxicities if any will be caused by the adenovirus. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive one intratumor injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU).

5.3 Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitation imposed by production of the adenovirus).

5.4 Dose Escalation: The adenovirus dose will increase in one-$\log_{10}$ increments for each group until 10^9 PFUs are reached. Patients entered at a given dose level will not be eligible for dose escalation. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients in each group (resectable and non-resectable).

5.5 All patients shall be registered with the Data Management office at At the time of registration, a prestudy form shall be necessary on each patient. When applicable, information pertaining to important prognostic factors such as tumor histology, pretreatment weight loss, performance status, disease stage and extent, and prior therapy will be requested.

5.6 Patients with local-regional tumors will have injection of a total dose of 10 ml for tumors ≥4 cm in diameter or 3 ml for tumor <4 cm in diameter of the adenovirus preparation with the appropriate number of viral particles at multiple sites percutaneously or transorally. Direct endoscopic injections may also be used is required. Injections will be placed at approximately 1 centimeter increments.

5.7 The treatment will be repeated three times weekly for two weeks. Dose escalation may proceed after a minimum two week follow-up of the last patient entered into the previous dose level. Treatment will continue on a monthly basis as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.

5.8 Patients will wear a surgical mask for 24 hours following injection of the adenovirus. All medical personnel will wear masks and face shields routinely during endoscopy and injection of the adenovirus. Anti-tissves will be prescribed as necessary. All patients will be kept in isolation during the time they are receiving injections of the adenovirus and for 48 hours after the last injection.

5.9 Those patients with surgically resectable disease will be treated by tumoral injection of adenovirus preparation as described in 5.6 and 5.7. The treatment will be repeated for two consecutive courses. Within four days of completion of the second course, the patients will be eligible to proceed with surgical resection. At the completion of surgical resection, prior to closure, 10ml of adenovirus preparation will be administered into the surgical defect (operative bed) and allowed to remain in contact for 60 minutes. The wounds are then closed and drains placed. Post-operatively, on the third post-operative day (prior to drain removal), 10ml of adenovirus preparation is sterily introduced into the drains and retrograde placed into the wounds and is allowed to remain for two hours. The drains are then replaced to suction and removed when indicated by the attending staff surgeon.

6.0 PRE-TREATMENT EVALUATION

6.1 A complete history and physical to include performance status, recent weight loss, usual weight and concurrent non-malignant disease and its therapy, and all prior anticancer treatments must be recorded.

6.2 Laboratory studies shall include quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, HIV analysis, urinalysis, and chest x-ray.

6.3 Any residual toxicity from prior therapies should be recorded using the grading schema in Appendix C.

6.4 Appropriate studies should be obtained to fully define the extent and severity of existing or suspected malignant and non-malignant disease.

6.5 Measurements of disease that can be measured on a C.T. scan of the head and neck will be documented. The location and size must be recorded prior to treatment. A photograph of the area

will be taken at a fixed distance from the lesion. The area will be measured in 2 dimensions (product of longest and perpendicular dimensions).

6.6 A pretreatment blood sample will be collected and stored for analysis of leukocytes and serum.

6.7 Biopsy specimens ,which have been previously obtained and have been confirmed for recurrent squamous cell of the head and neck, will be microdissected and analyzed for p53 mutations using SSCP analysis and direct DNA sequence analysis. The prescence or absence of p53 mutations will not, however, be an exclusion criteria for eligibility.

7.0 EVALUATION DURING STUDY

7.1 Patients will have a CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray prior to each course of therapy. Serum will be collected pre- and post-treatment for analysis of antibodies to adenovirus proteins.

7.2 History and physical with performance status and weight should be recorded before each course.

7.3 The tumors will be photographed clinically or endoscopically (depending upon site of disease) at the beginning of each course. Tumor measurements are to be recorded before each course for all measurable tumors.

7.4 All relevant information regarding viral dosage, tumor response, laboratory examinations, and treatment-related toxicities must be recorded before each treatment is given.

7.5 Parameters to be Measured In Vitro

7.5.1 Core biopsies or incisional biopsies will be obtained of accessible local and or regional tumor. Tumor specimens will be collected 72 hrs. after the last injection of the adenovirus during the first treatment cycle. Tissue will be fixed immediately in 4% paraformaldehyde and 0.5% glutaraldehyde at 4°C and 10% formalin for histopathologic verification and evaluation. This will permit extraction of DNA and RNA and permit in situ hybridization.

7.5.2 Biopsies will be analyzed for incorporation of the transduced gene into the host genomic DNA and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction. Pre- and post infected tissue biopsies will be evaluated histopathologically for any cytomorphologic changes. Tissue blocks will be retained for further evaluation of the p53 by immunohistochemistry and in-situ hybridization.

7.5.3 All patients will be evaluable for response and toxicity following one course of therapy.

7.6 An autopsy will be requested and immediately performed on all patients enrolled in the protocol who die. DNA will be extracted from tumor and normal tissues to determine if the adenovirus genes are present and expressed. PCR amplification of specific sequences for the adenovirus vector will be used to determine this. The following organ tissues will be analyzed in consented necropsies by PCR and routine light microscopy: upper aerodigestive tract mucosa , blood, brain, peripheral nerves (radial nerve), lung, liver, thyroid, adrenals, kidney, gastric, small bowel, and colonic mucosa, bladder mucosa, pancreas, gonad (testicles or ovaries, respectively), breast tissue, bone, pectoralis muscle, large and intermediate size vascular tissue (aorta and carotid) , and skin.

7.7 A blood sample will be collected three times at one-half hour intervals following injection of the adenovirus. These samples will provide leukocytes to analyze for uptake of adenovirus DNA. Serum will be tested for antibodies to adenovirus proteins. This will be done by western blot analysis performed by Microbiological Associates, Inc. (Rockville, MD). Patients will be tested monthly during treatment, monthly for the first three months following completion of treatment, every three months for the remainder of the year following completion of treatment, and then at least yearly thereafter. Urine and sputum samples are analyzed daily following virus treatment for adenovirus antibodies.

7.8 Normal tissue samples will be collected during the follow-up visits and endoscopies. These will include samples of non-malignant mucosa, lymphocytes, and germ cells, if possible. These tissues will be analyzed for incorporation of the adenovirus.

7.9 A staging CT scan of the head and neck to evaluate local and regional disease will be obtained on an every three month basis during treatment.

8.0 CRITERIA FOR RESPONSE AND TOXICITY

8.1 The graded toxicity scale used in this study has been previously described[29]. Three patients will be initially entered at each dose level in each group (resectable and non-resectable). If one patient in the cohort of 3 develops grade 3 toxicity for any system except hematologic (grade 4 required), an additional 3 patients will be entered at that dose level. If 2 of the 6 patients develop grade 3 (grade 4 hematologic) or greater toxicities, the next lower dose level not causing these toxicities is declared the Maximum Tolerated Dose (MTD). If MTD toxicity were to occur, patients could continue treatment at the next lower dose level. The MTD will be determined separately for each phase of the study.If MTD toxicity occurs in a cohort of 3 patients, then the next 3 patients may bypass the adenovirus alone phase and initially receive the adenovirus and cisplatin to establish the MTD for this combination.

8.2 The tumor bed and or neck will be photographed prior to each course of therapy for aerodigestive tract primary lesions.

8.3 The longest diameter and its perpendicular will be measured will be determined for measurable lesions. Size will be reported as the product of the diameters.

8.4 The rate of regrowth of the tumor will be calculated from the above measurements.

8.5 Patients will be evaluable for response if they have received at least one course of therapy. A complete response is defined as disappearance of all clinical evidence of tumor without the appearance of new lesions for a period of at least four weeks. Patients evaluable for a less-than-complete response must have had a bidimensionally measurable tumor. Partial response is defined as a 50% or greater reduction in the sum of the products of the diameters of the measurable disease; a minor response is defined as a 25% to less than 50% reduction in the sum of the products of the diameters of the measurable lesion. Patients are designated as having progressive disease if they show a 25% or greater increase in the size of their disease or if they develop unequivocal new lesions during treatment, and having no change if they have any tumor change not meeting the criteria described above.

8.6 The time to progression will be measured from the first observation with reduction in tumor bulk until there is evidence of progressive disease. Progressive disease is defined as an increase of $\geq$ 25% in the sum of the products of the diameters of the measured lesion. Patients must have received at least two courses of therapy before a designation of progression is made. The survival of patients will be measured from entry into protocol.

8.7 Alternative biologic endpoints will also be monitored. Pre-therapy and three days following the final treatment biopsies will be obtained and analyzed as described in section 7.5.2. Percentage of transfected cells in 3 random 100 x magnification fields will be determined. Maximal transduction rate will be determined by in-situ and immunohistochemically.

8.8 All toxicities encountered during the study will be evaluated according to the grading system (0-4) in Appendix C and recorded prior to each course of therapy. Duration of the toxicity and its treatment will be recorded. Life-threatening toxicities should be reported immediately to the Study Chairperson, who in turn, must notify the IRB, RAC, and FDA.

9.0 CRITERIA FOR DISCONTINUING THERAPY

9.1 Progression of obstructing airway tumor that has recurred after a minimum of 2 courses of treatment.

9.2 The development of unacceptable toxicity defined as unpredictable, irreversible, or Grade 4 (non-hematologic). Patient refusal of therapy due to a specific toxicity should be graded as 4 and an explanatory note recorded.

9.3 Non-compliance by patient with protocol requirements.

9.4 Patient refusal to continue treatment.

9.5 Criteria for removal from protocol:
- a) Refusal to continue study participation
- b) Significant hemoptysis
- c) Coagulopathy
- d) Progressive pneumonitis or other infectious processes.

10.0 DATA AND PROTOCOL MANAGEMENT

10.1 Protocol Compliance: The attending physician and oncology research nurse must see patients prior to drug administration. All required interim and pretreatment data should be available and the physician must have a designation as to tumor response and toxicity grade.

10.2 Data Entry: Data must be entered into the Clinical Data Management System before a course of therapy can be given. A brief explanation for missing data should be recorded as a comment.

10.3 Accuracy of Data Collection: The Study Chairperson will be the final arbiter of response of toxicity should a difference of opinion exist.

11.0 STATISTICAL EVALUATION

Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitations imposed by production of the adenovirus). A maximum of 21 patients will be entered into each study group, for a total of 42 patients for the entire study.

12.0 REFERENCES

1. Van der Riet, P., Nawroz, H., Hruban, R.H., Corio, R., Tokino, K., Koch, W., and Sidransky, D. Frequent loss of chromosome 9 p21-22 early in head and neck cancer progression. Cancer Res 1994; 54: 1156-1158.

2. Bishop JM. Molecular themes in oncogenesis. Cell 1991;64:235-248.

3. Hollstein M, Sidransky D, Vogelstein B, Harris CC. *p53* mutations in human cancers. Science 1991;253:49-53.

4. Lane DP, Benchimol S. *p53*: oncogene or anti-oncogene? Genes & Develop 1990;4:1-8.

5. Bressac B, Galvin KM, Liang TJ, Isselbacher KJ, Wands JR. Abnormal structure and expression of *p53* gene in human hepatocellular carcinoma. Proc Natl Acad Sci USA 1990;87:1973-1977.

6. Dolcetti R, Maestro R, Feriotto G, Pelucchi S, Rizzo S, Boiocchi M. *p53* genetic abnormalities in human squamous cell carcinomas of the larynx. Oncogene 1990;6:44-45.

7. Rodrigues NR, Rowan A, Smith MEF, et al. *p53* mutations in colorectal cancer. Proc Natl Acad Sci USA 1990;87:7555-7559.

8. Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the *p53* gene occur in diverse human tumor types. Nature 1989;342:705-708.

9. Maestro, R., Dolcetti, R., Gasparotto, D., Doglioni, C., Pelucchi, S., Barzan, L., Grandi, E., and Boiocchi, M. High frequency of *p53* gene alterations associated with protein overexpression in human squamous cell carcinoma of the larynx. Oncogene 1992; 7: 1159-1166.

10. Chung, K.Y., Mukhopadhyay, T., Kim, J., Casson, A., Ro, J.Y., Goepfert, H., Hong, W.K., Roth, J.A. Discordant *p53* gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. Cancer Res 1993; 53: 1676-1683.

11. Mukhopadhyay T, Cavender A, Tainsky M, Roth JA. Expression of antisense K-ras message in a human lung cancer cell line with a spontaneous activated K-ras oncogene alters the transformed phenotype. Proc Amer Assoc Cancer Res 1990;31:304.

12. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693-702.

13. Chen P-L, Chen Y, Bookstein R, Lee W-H. Genetic mechanisms of tumor suppression by the human *p53* gene. Science 1990;250:1576-1580.

14. Yonish-Rouach E, Resnitzky D, Rotem J, Sachs L, Kimchi A, Oren M. Wild-type *p53* induces apoptosis of myeloid leukemic cells that is inhibited by interleukin-6. Nature 1991;352:345-347.

15. Shaw P, Bovey R, Tardy S, Sahli R, Sordat B, Costa J. Induction of apoptosis by wild-type *p53* in a human colon tumor-derived cell line. Proc Natl Acad Sci USA 1992;4495:4499.

16. Fujiwara T, Grimm EA, Mukhopadhyay T, Cai DW, Owen-Schaub LB, Roth JA. A retroviral wild-type *p53* expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis. Cancer Res 1993;53:4129-4133.

17. Lowe SW, Ruley HE, Jacks T, Housman DE. *p53*-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993;74:957-967.

18. Merino OR, Lindberg RD, Fletcher GH. An analysis of distant metastasis from squamous cell carcinoma of the upper respiratory and digestive tracts. Cancer 1977; 40: 147-156.

19. Votova C Jr, Fletcher GH, Jesse RH Jr, Lindberg RD. Management of cervical nodes, either fixed or bilateral, from squamous cell carcinoma of the oral cavity and faucial arch. Radiology 1972; 105: 417-420.

20. Komaki R, Garden AS, Cundiff JH. Endobronchial Radiotherapy. In: Roth JA, Hong WK, Cox JD, eds. Advances in Lung Cancer. Cambridge,MA: Blackwell Scientific Publications,Inc., 1992:

21. Boshart M, Weber F, Jahn G, Dorsch-Hasler K, Fleckenstein B, Schaffner W. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 1985;41:521-530.

22. Mcgrory WJ, Bautista DS, Graham FL. A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. Virology 1988;163:614-617.

23. Graham FL, Eb VD. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 1973;52:456-467.

24. Zhang WW, Fang X, Branch CD, Mazur W, French BA, Roth JA. Generation and identification of recombinant adenovirus by lipsome-mediated transfection and PCR analysis. BioTechniques 1993;

25. Graham FL, Prevec L. Manipulation of adenovirus vectors. In: Murray EJ, ed. Methods in Molecular Biology, Gene Transfer and Expression Protocols. New Jersey: The Humana Press Inc, 1991:109-128.

26. Dai Y, Roman M, Naviaux RK, Verma IM. Gene therapy via primary myoblasts: long-term expression of factor IX

protein following transplantion in vivo. Proc Natl Acad Sci USA 1993;89:10892-10895.

27. Georges RN, Mukhopadhyay T, Zhang YJ, Yen N, Roth JA. Prevention of orthotopic human lung cancer growth by intratracheal instillation of a retroviral antisense K-ras contruct. Cancer Res 1993;53:1743-1746.

28. Casson AG, Mukhopadhyay T, Cleary KR, Ro JY, Levin B, Roth JA. p53 gene mutations in Barrett's epithelium and esophageal cancer. Cancer Res 1991;51:4495-4499.

29. Ajani JA, Welch SR, Raber MN, Fields WS, Krakoff IH. Comprehensive criteria for assessing therapy-induced toxicity. Cancer Invest 1990;8 (2):147-159.

Appendix A: Evaluation Before and During Therapy

Appendix B: Zubrod Scale of Performance Status

Appendix C: Toxicity Criteria

Appendix D: Figures/Figure Legends

Appendix E: Biosafety Procedures

Appendix F: Informed Consent

APPENDIX A
EVALUATION BEFORE AND DURING THERAPY

	Pre-Study	After completion of each course*	At two week intervals	Before each subsequent course	After completion of 1st course and every 3 mos
History	x	x	x	x	
PE	x	x	x	x	
Staging examination					
C.T. examination	x				x
Tumor size and response	x	x	x	x	
CBC, diff,	x	x	x***	x	
platelets, PT/PTT	x			x	
SMA-12	x	x	x***	x	
Serum Electrolytes	x	x	x	x	
Chest x-ray	x	x	x	x	
Toxicity and perf. status notation	x	x	x	x	
Completion of protocol specific template on Data Management System**	x	x	x	x	

* Any studies necessary to completely evaluate malignant and concurrent non-malignant diseases and drug toxicity must be obtained and recorded at baseline and before each course as appropriate.

** Any studies requested or added to protocol-specific data template of Clinical Data Management system must be collected as specified.

*** Weekly if indicated

Appendix B
Performance Status Scales

Karnofsky Performance Scale (1)

Point	Description
100	Normal; no complaints; no evidence of disease
90	Able to perform normal activity; minor signs/symptoms of disease
80	Normal activity with effort; some signs/symptoms of disease
70	Cares for self; unable to perform normal activity or do active work
60	Requires occasional assistance, but is able to care for most of personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled; hospitalization indicated; death not imminent
20	Very sick; hospitalization and active supportive treatment necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

Zubrod Performance Scale (2)

Point	Description
0	Normal activity; asymptomatic
1	Symptomatic; fully ambulatory
2	Symptomatic; bed <50% of time
3	Symptomatic; in bed 50% of time; not bedridden
4	100% bedridden
5	Dead

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Protocol Title: **Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wild-type p53**

1. _____ _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so that you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other consents you may have signed in other protocols.

DESCRIPTION OF RESEARCH

2. PURPOSE OF THE STUDY: Some cancers that occur in the head and neck area may be due to a defect in a gene called p53. The purpose of this clinical research study will be to see whether a normal copy of the p53 gene can be placed inside the patient's cancer cells and cause the cancer to grow more slowly or to stop growing.

3. DESCRIPTION OF RESEARCH: To introduce the normal p53 gene into tumor cells utilizing a defective virus of the adenovirus type which is similar to other viruses that cause the common cold. This defective virus is called a "vector". These tumors will be injected directly with the adenovirus three times a week for two weeks. After two weeks of rest from receiving treatment, tumors will then be injected again with the virus three times a week for two consecutive weeks. If the tumor can be removed in its entirety, surgery will be performed for removal of tumor that is considered completely removable by the patient's physician. Surgery must be performed within four days after completion of the last injection of virus. At the time of surgery, additional virus is placed into the area where the tumor has been removed. Tubing, which is ordinarily placed in surgical areas, is used to allow for drainage of fluids from the surgery area. Three days after the tumor has been removed, virus will be placed into the tubing and allowed to enter the surgical site once again. If the patient has undergone surgery, this will be the last treatment with adenovirus.

If the cancer cannot be removed by the physician, this tumor will be injected directly

three times a week for two consecutive weeks. After two weeks of rest from treatment, courses of three injections weekly for two consecutive weeks are repeated on a monthly basis. Injections of adenovirus are continued if tumor continues to shrink. If there is absence of tumor shrinkage, evidence of tumor growth, or adverse reactions to the adenovirus injections, treatment will be terminated.

The injections into the tumor are delivered with a skinny needle. If the tumor is greater than approximately two inches in size, approximately two teaspoons of virus will be injected into the tumor. Smaller tumors, less than two inches, will be injected with less than one teaspoon of virus. Multiple injections of virus into the tumors will be required with these injections being spaced apart by approximately one-half inch each. Numbing medication may be placed on the overlying skin to decrease discomfort from injections.

Patients with tumors of the throat or the voice box may also receive the treatment. It may be necessary to remove a part of the tumor surgically or with a laser before the treatment with p53 is given. Other patients may require that a tracheostomy is first performed. A tracheostomy is a surgical operation to make an opening into the windpipe. Certain routine diagnostic studies will be performed before entry into this trial. These involve local examination of the tumor by inserting an instrument with a light into the throat.

If previous specimens are insufficient for laboratory studies related to this research, additional biopsies will be needed. The treatment will be repeated monthly as long as there is evidence that the tumor is not growing.

The experimental treatment and costs related to the patient's participation in this research and which include clinical examinations, biopsies, and other forms of testing will be provided free to the patient. A maximum of 42 patients will be entered in this study. Twenty one patients may have injections of adenovirus with surgery and twenty one patients may have injections of adenovirus without surgery.

The patient's course will be followed indefinitely. Dr. Clayman's office should be notified if an address change is made.

PERMISSION FOR AUTOPSY: In case of death, the family of the patient will be asked for permission to perform an autopsy.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

Two small additional biopsies will be required in addition to the initial biopsy. Risks from biopsies include coughing up blood which is usually slight. Severe hemorrhage which requires emergency treatment is rare. Biopsy of neck masses may also be associated with a slight risk of bleeding or infection. This clinical research study may involve unforeseeable risks to the participant.

Possibility of Causing a New Cancer. It is possible that the research could cause cancer in normal cells although this risk is believed to be small when the injected virus has been properly safety tested prior to its use. The adenovirus vector has only been used, to date, on the lining of the breathing tube in over twenty patients with no ill effects noted.

4a. This clinical procedure may involve unforeseeable risks to the unborn children, therefore, the participants should practice adequate methods of birth control throughout the period of their involvement in the clinical study if they are sexually active. To help prevent injury to children, the female participants should refrain from breast feeding during participation in the clinical research study.

5. POTENTIAL BENEFITS:

This treatment may result in shrinkage of the tumor, which may decrease cancer associated symptoms or may prolong life.

6. ALTERNATE PROCEDURES OR TREATMENTS:

Chemotherapy or other experimental drugs may be an alternative for some individuals. These treatments cause shrinkage of cancer in a number of patients. Another option is to only control the symptoms of disease and not take chemotherapy treatment.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the procedure involved and the investigator has been willing to reply to my inquiries. This procedure will be administered under the above numbered, title, and described clinical research protocol at this institution. I hereby authorize Dr. _____ the attending physician/investigator and designated associates, to administer this procedure.

8. I have been told and understand that my participation in this clinical research study is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences with

effects of my decision with my physician.

In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

I will be informed of any new findings developed during the course of this clinical research study which might be relevant to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration, Microbioiogical Associates, Magenta Corporation (manufacturers of the virus), and National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that, should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available for treatment at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for such injury.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost.

Costs related to my medical care will be covered by the Introgen Sponsored Research Agreement. Clarification of specific cost issues may be addressed in section 3 of this informed consent. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, devices, new drugs, or possible patentable procedures, in which event I understand that I cannot expect to receive any compensation or benefits from the subsequent use of information acquired and developed through my participation in this research project.

13. I understand that refraining from breast feeding and practicing effective contraception is medically necessary and a prerequisite for my participation in this clinical research

study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.

14. I may discuss any questions or problems during or after this study with Dr. Gary L. Clayman at In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman or the Surveillance Committee at and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based upon the above, I consent to participate in the research and have received a copy of the consent form.

DATE	SIGNATURE OF PARTICIPANT
WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR	SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

I have discussed this clinical research study with the Participant and/or his or her authorized representative using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe the participant understood this explanation.

PHYSICIAN/INVESTIGATOR

Appendix C
Toxicity Criteria

	Grade 1	Grade 2	Grade 3	Grade 4
ALLERGY				
ACUTE ALLERGIC REACTION	Transient rash, Drug fever <38C/100.4F	Urticaria, Drug fever >38C/100.4F mild bronchospasm	Serum sickness, bronchospasm, req parenteral meds	Anaphylaxis
FEVER WITH DRUG	<38C, <100.4F	38-40c, 100.4-104F	>40C/>104F despite antipyretic rx	Fever & hypertension
CARDIOVAS-CULAR.				
ARRHYTHMIA	Resting sinus tech, PAC's, <1 PVC/hr. nonspec S-T or wave abn, 1st (dog) AV block	Sustained atrial arrhythmia, 1-9 PVC, hrs. ischium ST or T wave, Mobitz type I, incompl or rate-related bundle branch block	10-20 PVC/hr. multifocal PVC couplets, 3-5 consec PVC and salvon, ing pattern on EKG, Mobitz type II, Bundle branch or bifascic block	>30 PVC/hr. 6 consec PVC MI, 3rd dec AV block
CARDIAC SYMPTOMS	Mild or transient	Symptoms on exertion	Symptoms at rest, persistent	Severe symptoms, non-response to rx
CARDIAC BIOPSY	0.5	1.0	1.5	>1.5
EJECTION FRACTION ABNORMALITY	>60-64%	50-59%	40-49%	<40%
HI TENSION	10-20% dec systol	21-30% dec systol	31-40% dec systol, req pressors	>40% dec systol, not response to press
PERICARDIAL EFFUSION	Small	Moderate	Large, no tamponade	Large, tamponade
PERIPH CAPILLARY LEAKAGE	Min ankle pitting edema	Ankle pitting edema & wt. gain <10 lbs.	Periph edema, wt. gain >9.9 lbs, pleural effusion w/no pul fx deficit	Anasarca, sev pleural effusion w/pul rx deficit, asc

	Grade 1	Grade 2	Grade 3	Grade 4
CNS				
AFFECT ABNORMALITY	Transient panic/apathy	Sustained panic/apathy	Sustained panic/apathy, rx req	Sustained panic/apathy nonresp to rx
ATAXIA	Mild/transient gait or limb	Mod gait or limb ataxia	Mod gait & limb ataxia	Disabling ataxia,
AUTONOMIC DYSFUNCTION	Abnormal sweating	Impotence	Asympt arrhythmia, orthostat hypotension	Sympt arrhythmia, orthostat hypotensi
BLADDER DYSFUNCTION AUTONOMIC	- - - -	Dysfunction not req cath	Req cath	Req permanent cath
COGNITIVE DEFICIT	Slow, accurate	Impaired memory/new learning	Global deficiency	Unresponsive
CONSTIPATION AUTONOMIC	Mild, no rx req	Occasionally req cathartics	Daily cathartics/enema req	Abdominal distention, vomitin
LEVEL OF CONSCIOUSNESS	Drowsy, easily arousable	Response to loud or tactile stimuli	Response to pain only	Unresponsive to any stimuli
FOCAL SEIZURES	Isolated	<3/day	>2/day	Status epilepticus
GENERALIZED SEIZURES	Isolated	<3/day	>2/day	Epilepsia partialis continue
HEARING LOSS	Transient decrease, tinnitus	Moderate loss	Severe loss	Deaf
LANGUAGE ABNORMALITY	Word finding difficulty	- - - -	Reduced verbal output or comprehension	Global aphasia
MOTOR DEFICIT	Mild/transient weakness	Mod weakness, ambulatory	Non-ambulatory	Complete paralysis

	Grade 1	Grade 2	Grade 3	Grade 4
MOVEMENT DISORDERS	Transient and limb movement	Mod limb/gait disorder	Severe and reversible parkinsonism, dystonia or tremor	Permanent parkinsonism tremor or dystonia
PERCEPTION ABNORMALITY	Occasional misperception	Brief unformed hallucination	Freq formed hallucination	Constant hallucinat
SENSORY DEFICIT	Paresthesias, decr DTSs	Mild objective abnormality, absent DTRs	Severe paresthesia, mod objective abnormality	Complete sensation
SPEECH ABNORMALITY	Mildly slurred	Moderate slurring	Unintelligible	Mute
VERTIGO	Mild, transient	Moderate, nausea	Associated w/N&V	Disabling, intractab
VISION	Slight reduced acuity	Finger counting only	Light perception only	Blind
DERMATOLOGIC				
ALOPECIA	Partial loss	Complete loss	Non-reversible loss	
CHELITIS	Chapping	Fissures	Bleeding	Necrosis
SKIN REACTION	Local erythema, dry skin, mild or transient rash	Diffuse erythema, dry desquamation, macropap rash, pruritus	Moist desquamation, ulceration, bullous disease	Exfoliative dermatitis, necrosis, req surg
GASTROINTES-TINAL				
DIARRHEA	Transient, <2 days	Tolerable, >2 days	Intolerable, req/s rx	Hemorrhagic, dehydrat
NAUSEA AND VOMITING	Mild nausea alone	Mod nausea, transient vomiting	Severe nausea or vomit, req's rx	Intract vomiting req hosp.

	Grade 1	Grade 2	Grade 3	Grade 4
STOMATITIS	Soreness, erythema	Erythema, patchy, ulceration can eat solids	Confluent ulceration, liquid diet	Hemorrhagic ulceration, necrosis, req parenteral support
GENERAL				
ACHING PAIN	Mild	Moderate	Severe	Intractable
CHILLS	Mild	Moderate	- - - -	- - - -
HEMATOLOGIC				
ANEMIA	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
GRANDULO-CYTOPENIA	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
HEMO-RRHAGE	Pepechise, min blood loss, no trans req	Blood loss req 1-2 U trans	Blood loss req 3-4 U trans	Blood loss req <4 U trans
LEUKOPENIA	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
THROMBO-CYTOPENIA	75-99	50-74	25-49	<25
HEPATIC				
ALK PHOS	1.5-2.5 x N, MDACC 165-275	2.6-5 x N, MDACC 276-550	5.1-20 X N, MDACC 551-1100	>10 x N, MDACC >1100
BILIRUBIN INCREASE	1.5-2.5 x N, MDACC 1.5-2.5	2.6-5 x N, MDACC 2.6-5	5.1-20 x N, MDACC 5.1-10	>10 x N, MDACC >10
HEPATIC SYMPTOMS	- - - - - -	- - - - - -	Precoma	Hepatic coma
TRANSAMINASE INCREASE	1.5-2.5 x N, MDACC SGOT 45-75, SGPT (SMA) 60-100	2.6-5 x N, MDACC SGOT 76-150, SGPT (SMA) 101-200	5.1-20 x N, MDACC SGOT 151-300, SGPT (SMA) 201-400	>10 x N, MDACC SGOT >300, SGPT (SMA) >400
INFECTION				
INFECTION	FUO	Minor infection	Major organ infection	Disseminated infection
PULMONARY				
FVC FUNCTION ABNORMALITY	FVC 70-80% pred, FEV1 or DLCO 60-80% pred, 15-25% dec from abn baseline	FVC 50-69% pred, FEV1 or DLCO 40-59% pred, 26-50% dec from abn baseline	FVC <50% pred, FEV1 or DLCO >40% pred, >50% dec from abn baseline	Unable to perform test due to resp dist
RESPIRATORY SYMPTOMS	Mild or transient	Symptoms on exertion	Symptoms at rest, persistent	Severe symptoms, nonresponse to rx
RENAL				
CREATININE	<1.25 x baseline	1.25-2.5 x baseline	2.6-5 x baseline	>5 x baseline
DYSURIA	Mild	Moderate	Severe	Unacceptable
HEMATURIA	6-10 RBC/HPF	11-50 RBC/HPF	Gross, >50 RBC/HPF	Clots, obstructive
PROTEINURIA	1+, <0.3g%, <3g/L	2-3+m 0, 3-1, 0g%, 2-10g/L	4+, >1.0g%, >10g/L	Nephrotic syndrome

Appendix D

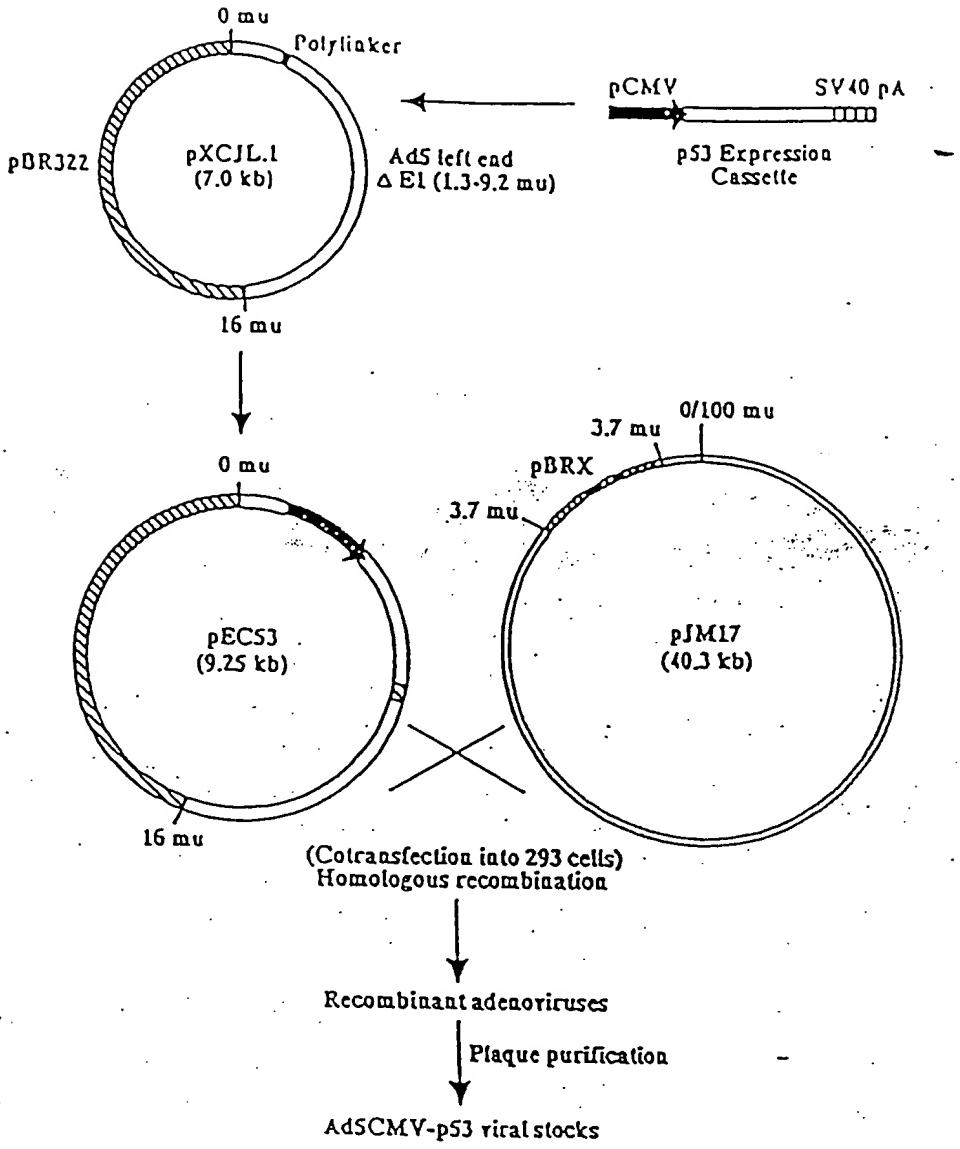

Figure 1. Scheme for generation of recombinant p53 adenovirus. The p53 expression cassette was inserted between the Xba I and Cla I sites of pXCJL.1. The p53 expression vector (pEC53) and the recombinant plasmid pJM17 were cotransfected into 293 cells. The transfected cells were maintained in medium until the onset of the cytopathic effect. Identification of newly generated p53 recombinant adenoviruses (Ad5CMV-p53) by PCR analysis of the DNA samples prepared from the cell culture supernatants was described elsewhere[24].

Appendix D

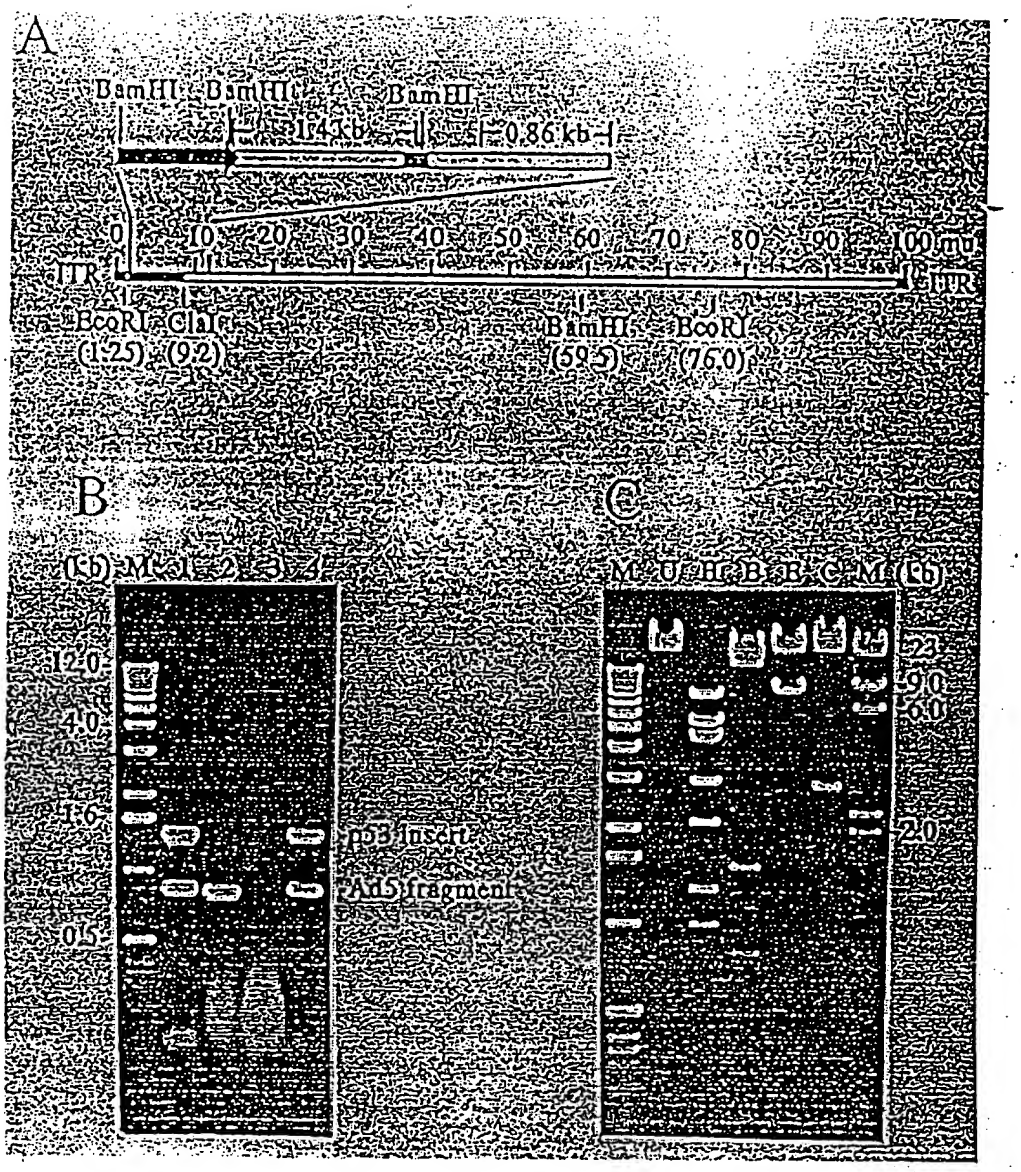

Figure 2. Structural analysis of Ad5CMV-p53 DNA. (A) A map of Ad5CMV-p53 genomic DNA, showing the locations of the p53 expression cassette, the PCR primers, and the restriction sites. (B) An agarose gel analysis of PCR products. Two pairs of primers that define 1.4-kb (p53) and 0.86-kb (Ad5) DNA fragments were used in each reaction. DNA templates used in each reaction were pEC53 plasmid (lane 1), Ad5/RSV/GL2 DNA (lane 2), no DNA (lane 3), and Ad5CMV-p53 DNA (lane 4). (C) Restriction mapping of Ad5CMV-p53 DNA. CsCl-gradient purified Ad5CMV-p53 DNA samples were digested with no enzyme (U), Hind III (H), Bam HI (B), Eco RI (E), and Cla I (C), respectively, and analyzed on 1% agarose gel.

Appendix D

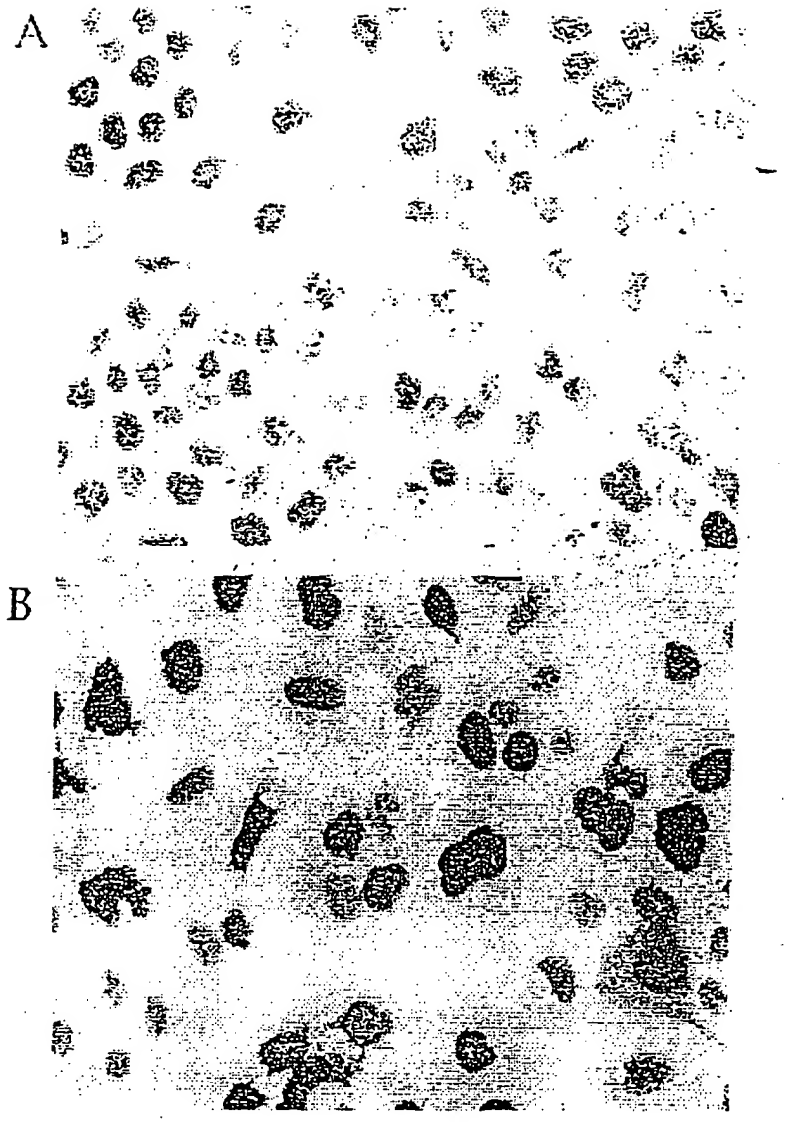

Figure 3. Infectivity of Ad5CMV-p53 in Tu138 cells. Tu138 cells were infected with Ad5CMV-p53 in dose response experiments or Ad5/RSV/GL2 at 50 PFU/cell for 24 h. Medium alone was used as a mock infection. The infected cells were analyzed by immunostainings. No differences in staining were noted comparing mock infection or Ad5/RSV/GL2. Absence of non-specific staining was confirmed by preabsorption methods. (A) Mock infection probed with anti-p53 antibody. (B) Ad5CMV-p53 infection probed anti-p53 antibody. The anti-p53 antibody used was PAb 1801, with the avidin-biotin method for staining.

Appendix D

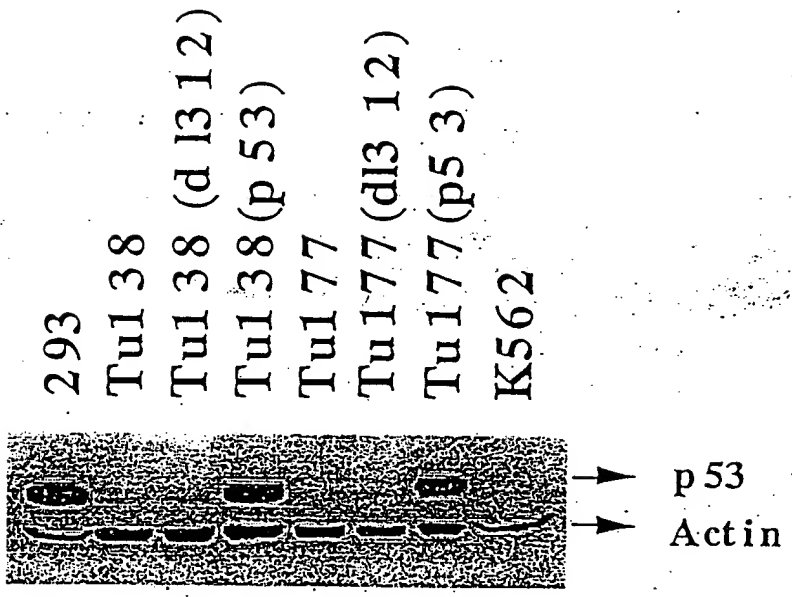

Figure 4. Relative level of expression of exogenous p53 in Tu138 and Tu177 cells. Samples of cells that were infected with Ad5CMV-p53 or Ad5/RSV/GL2 at 50 PFU/cell were prepared 24h after infection. The relative levels of expression of p53 were analyzed by Western blotting using anti-p53 and anti-actin antibodies. (A) Coomassie blue staining of an SDS-PAGE analysis, showing relative quantities of protein samples loaded. Western blot analysis 24-h post infection. Cell extracts isolated from cells were subjected to SDS-PAGE, blotted onto Hybond-ECL membranes, and probe with both anti-p53 and anti-actin antibodies. Lanes 1 and 8 are 293 and K562 cells, respectively. Lanes 2 and 5 are mock-infected Tu-138 and Tu-177 cells. Lanes 3 and 6 are Tu-138 and Tu-177 cells infected with replication-deficient adenovirus. Lanes 4 and 7 are Tu-138 and Tu-177 cells infected with Ad5CMV-p53.

Appendix D

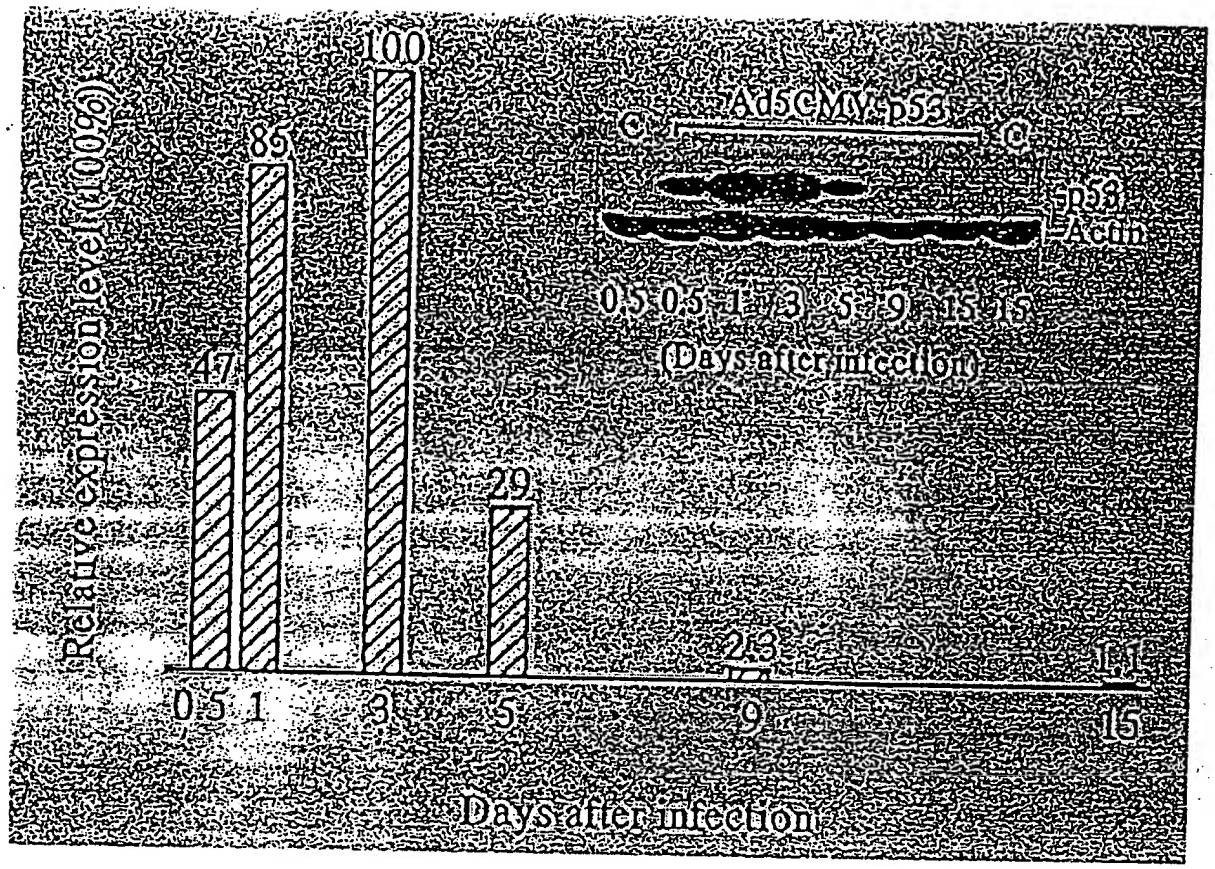

Figure 5. Time course of exogenous p53 expression in H358 cells. Multiple dishes of H358 cells were infected with Ad5CMV-p53 at 10 PFU/cell. Cell lysates were prepared at indicated time points after infection. Western blotting was probed with anti-p53 and anti-actin antibodies. Relative quantities of exogenous p53 were determined by densitometer.

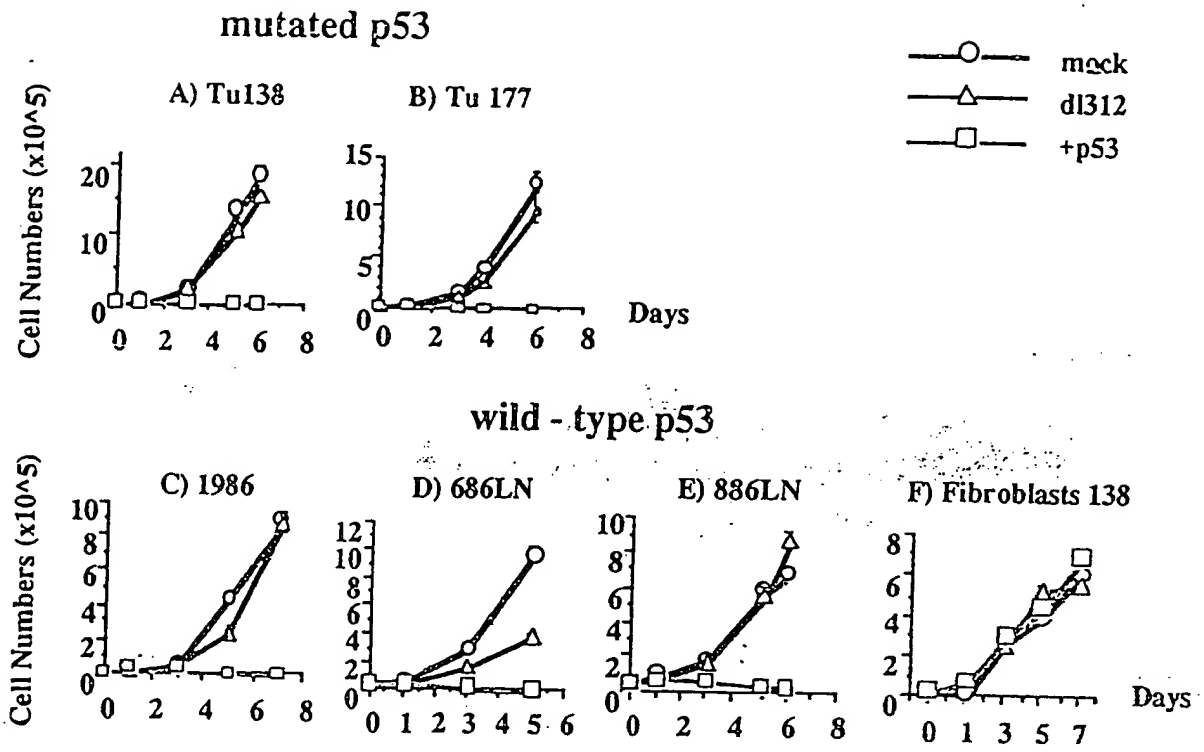

Figure 6. Growth curves of the Ad5CMV-p53-infected human head and neck squamous carcinoma and fibroblasts cells. The cells were inoculated at densities of $1x10^5$ in 60 mm culture dishes 24 h before infection. The cells were infected with Ad5CMV-p53 or Ad5/RSV/GL2 at 50 PFU/cell. Culture medium alone was used as mock infection. Triplets of each cell line for each treatment were counted daily from postinfection day 1 to day 6. The curves are plotted from the combined data of three experiments.

A) Tu138 B) Tu177 C) 1986 D) 686LN E) 886

F) Fibroblasts derived from Tu138 patient. Graphs A through E showed no viable tumor cell lines following 72 hours whereas non-malignant fibroblasts reveal normal growth (and morphology) characteristics.

Appendix D

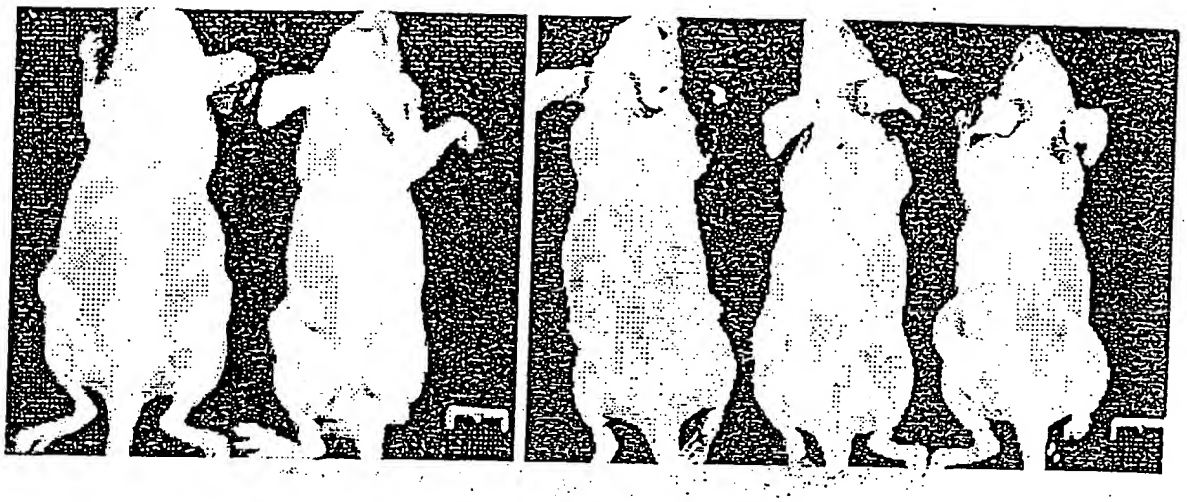

Figure 7. Inhibition of SCCHN cell growth *in vivo*. Pictures of the representative nude mice studies for both Tu1-38 (left) and Tu-177 (right) cell lines. The effect of Ad5CMV-p53 on established subcutaneous tumor nodules was determined in nude mice in a defined pathogen free environment. Experiments were reviewed and approved by institutional committees for both animal care and utilization and for recombinant DNA research. Briefly, following induction of acepromazine/ketamine anesthesia, three separate subcutaneous flaps were elevated on each animal and 5 x 10^6 cells in 150 µl of complete media were injected subcutaneously into each flap using a blunt needle; the cells were kept in the pocket with a horizontal mattress suture. Seven animals were used for each cell line. After 4 days, the animals were re-anesthetized and the flaps were re-elevated for the delivery of 100 µl of 1) Ad5CMV-p53 (10^8 PFU) in the right anterior flap; 2) replication-defective virus (10^8 PFU) in the right posterior flap; and 3) transport medium alone, in the left posterior flank. All injection sites had developed subcutaneous visual and palpable nodules before treatment was administered. Animals were observed daily and sacrificed on day 20.

Appendix D

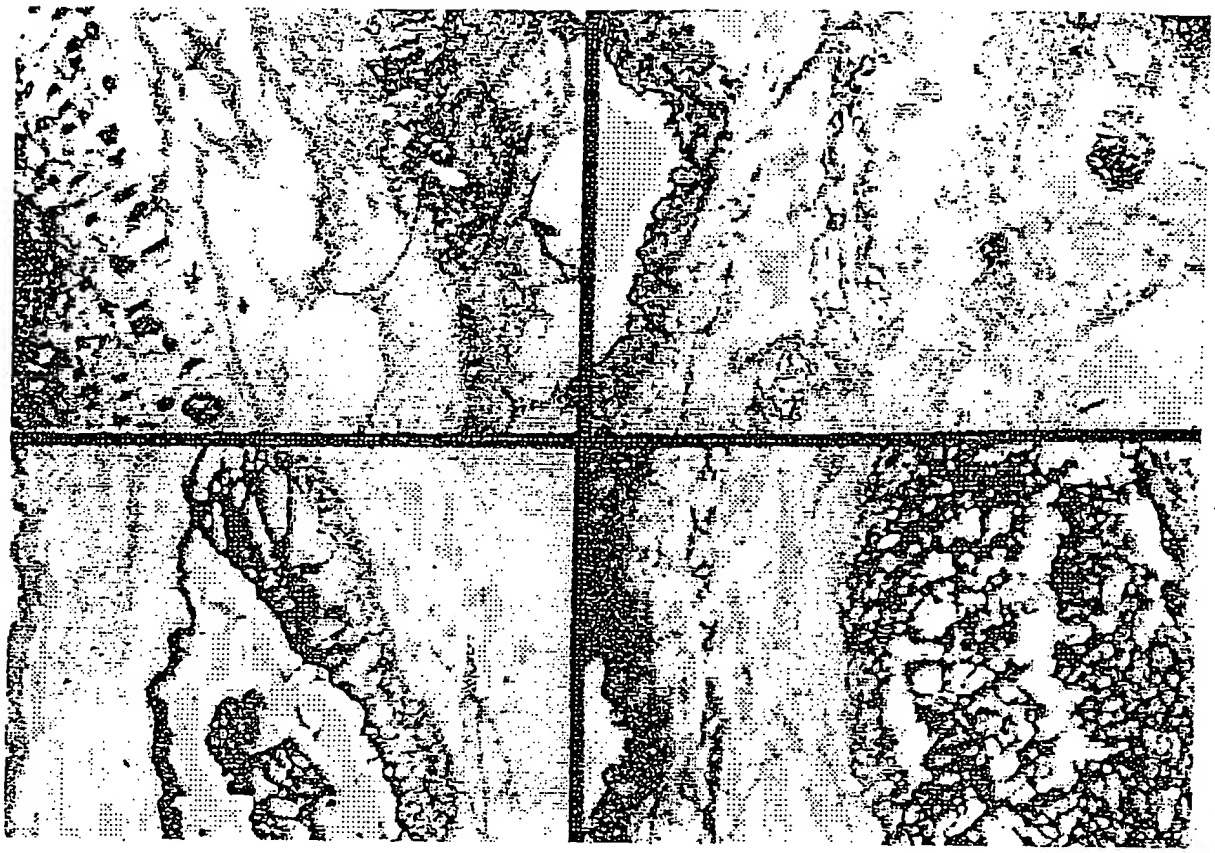

Figure 8. Dose response in-vivo infecting experiments in microscopic residual disease flap model. A marker virus AdCMVß-galactosidase exhibits blue X-gal staining within infected cells. Top left panel - Mock infection, Top right panel - 10^7PFU/cell, Bottom left panel - 10^8 PFU/cell, Bottom right panel - 10^9 PFU/cell. A clear dose-response is noted. Inflammation and edema increase histologically with increasing viral titers. Magnification 63 X.

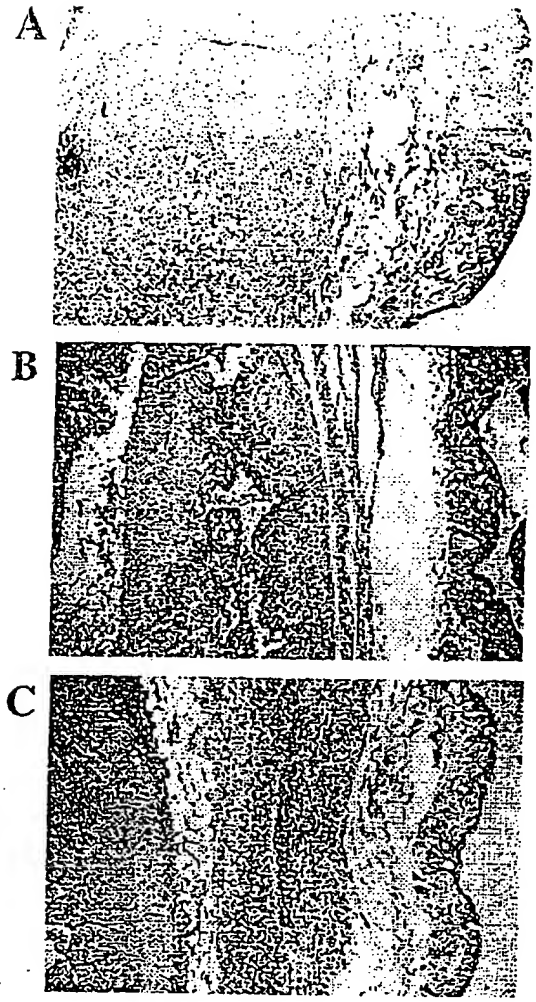

Figure 9. In-vivo infectivity of AdCMVp53 in microscopic residual disease flap model. The AdCMVp53 was pippetted into the subcutaneous flap 48h following tumor cell line delivery. A representative experiment of the wild- type p53 HNSCC cell line MDA686LN is shown. Panel A - mock infection showing lack of immunostaining in the wild-type p53 cell line, Panel B - 10^8 PFU/cell, Panel C - 10^9 PFU/cell. Immunostaining was performed using the polyclonal rabbit anti-human antibody OM1 (Signet Laboratories, Denham, MA) and the Avidin-biotin method. Basal immunostaining with viable tumor is seen in Panel A. Panel B shows peripheral tumor necrosis with immunostaining of in the more central portion of the tumor. Panel C reveals total necrosis of the tumor with immunostaining found in the entire surgical pocket with mutliple layers expressing protein including stroma and superfiscial muscular layers. Magnification 100X.

Appendix D

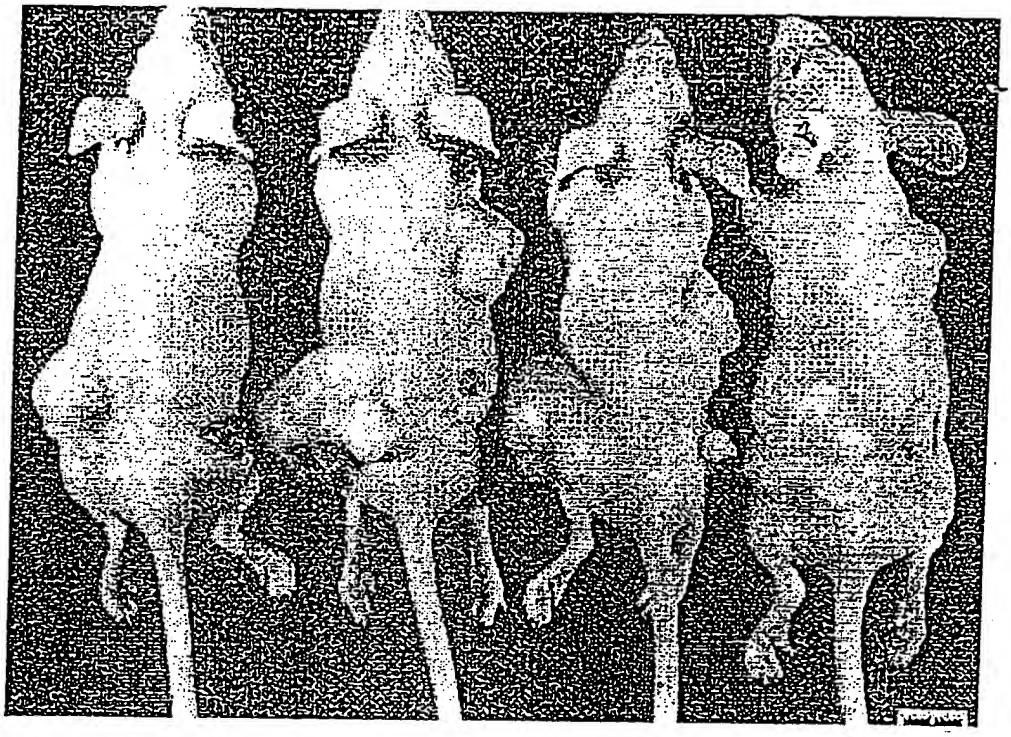

Figure 10. **Repeat in-vivo infection with AdCMVp53 on eastablished tumors in nu/nu female mice.** Established subcutaneous Tu138 tumors were allowed to develop to no greater than 1.5cm in greatest dimension in both right and left posterior flanks. Prior to the institution of adenovirus treatment there were no significant differences in tumor sizes in the posterior flanks. The left flank was peritumorally infiltrated with 10^9 PFU of dl 312 in 0.1 ml and the right flank with AdCMVp53 at the same dosage (10^9 PFU). The animals were sacrificed due to tumor burdens and the above photograph taken. One animal was sacrificed earlier due to excessive tumor burden in the dl 312 site. A significant reduction in tumor mass is noted in the right flank. With reduction in tumor mass in the p53 treated sites, all animals developed skin loss and eschar formation. All animal experiments and care were performed in accordance with the University of Texas M.D. Anderson Cancer Center Animal Care and Use Committee.

5

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference: BB-IND 6165

AUG 2 4 1995

The University of Texas M.D. Anderson Cancer Center
Attention: Leonard Zwelling, M.D.
Associate Vice President, Clinical & Translational Research

Dear Dr. Zwelling:

We have reviewed your **Investigational New Drug Application (IND)** for "Adenovirus Vector (Ad5CMV-p53, M.D. Anderson) Expressing Wildtype p53 Gene; Administered Intralesional or via Tumor Bed," and as relayed to you in our letter of your study may proceed; however, we have the following comments and requests for additional information:

CLINICAL INFORMATION

1. Although the target population would appear to be patients who have failed conventional therapy, the eligibility criteria could be interpreted more broadly. It seems that patients who have residual disease following neoadjuvant irradiation or combined modality therapy are eligible for this protocol. Although these patients are not likely to have a durable complete response to conventional treatment, the informed consent should state more clearly that this is an experimental therapy, and that conventional surgical treatment is a reasonable alternative. Please submit the revised informed consent.

2. The protocol does not provide a clear definition of a treatment course. If injections administered three times per week for two weeks constitute a single course of treatment, this is stated clearly only in the informed consent. It is not clear if patients with multiple sites of disease will receive a fixed total dose of Ad5CMV-p53 equally divided between each injected site or if the total dose will vary according to the number of sites injected. If the latter is the case, then systemic exposure to the vector would increase with the number of sites. Please clarify the total and/or maximum dose of vector for each cohort.

3. A basic assumption of the protocol is that there will be no treatment related toxicity. We note that only grade 4 toxicity prompts changes in the treatment plan. We strongly recommend that the administration of the vector be discontinued or decreased in the event of a grade 3 or greater toxicity or any toxicity which does not resolve to less than or equal to grade 1 toxicity by the next treatment course.

4. Dose escalation in cohorts above 1 x 10^9 pfu should proceed at half-log increments rather than one log as currently specified in the protocol since doses at or above 1 x 10^9 pfu are associated with significant local toxicity. A clinical trial in patients with nonsmall cell lung cancer of very similar design is also being conducted at your institution. If you have any information regarding the toxicity in the ongoing clinical trial in patients with lung cancer at doses above 1 x 10^9 pfu which you feel may support a more aggressive dose escalation schema, please submit these data. In lieu of such data, we request that you submit a revised protocol with the modified dose escalation schema and plan for dose modification.

If you have any questions, please contact Jeanne Delasko at

Sincerely yours,

Sharon T. Risso

Sharon T. Risso
Director
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Revisions

6

approved
AUB
10-2-95
Aman U Buzdar MD

INTEROFFICE MEMORANDUM

RECEIVED
SEP 26 1995
Office of Protocol Research

TO: Aman U. Buzdar, M.D.
Chairman, Surveillance Committee

FROM: Gary L. Clayman, D.D.S., M.D. GLC
Department of Head and Neck Surgery

DATE: September 25, 1995

SUBJECT: Administrative Approval of Revised Protocol HNS 94-001 entitled, **"Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"**

The FDA has mandated that an additional change be added to Protocol HNS 94-001. This change includes clarification of dose escalation in cohorts above 1 x 10^9 pfu.

NOTE:

Treatment Plan:

5.4 Dose Escalation: The adenovirus dose will increase in one $\log_{10}$ increments for each group until 10^9 PFUs are needed. Thereafter, adenovirus doses will increase at half-log increments. Patients entered at a given dose level will not be eligible for dose escalation. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients in each group (resectable and non-resectable).

This modificiation is included in the revised protocol. I am requesting administrative approval for this change. The revised protocol must be submitted to the FDA within 21 days.

GLC:lkm

Attachment

✓cc: Leonard Zwelling, M.D.
Michael J. Keating, M.D.

PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title) (Two lines not to exceed 75 characters per line)

Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53

Study Chairman: Gary L. Clayman, D.D.S., M.D., Department of Head and Neck Surgery

Patient Eligibility: (Twenty lines not to exceed 75 characters per line)

1. Patients must have histologic proof of squamous cell carcinoma of the head and neck. Patients must be either unable to receive conventional treatment (e.g. the patient received radiation therapy with or without surgery) or have failed conventional treatment. Those patients with extensive local or regional disease that have persisted or recurred following radiation therapy (with or without chemotherapy or surgery) and have clinically resectable, but likely non-curable (<10% disease free survival) are also eligible. Patients need not have received a trial of chemotherapy prior to entering this protocol. All eligible patients will be discussed at the Head and Neck Surgery Multidisciplinary Treatment Planning Conference prior to protocol enlistment.
2. Patients must have clinical evidence of advanced local and/or regional cancer which is unresectable or for which no meaningful resection with surgical margins will be obtainable.
3. All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).
   All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.
5. Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative. Patients with upper respiratory infections will not be treated until the infection resolves.
6. Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of $>2{,}000/mm^3$ and platelet count of $100{,}000/mm^3$), adequate liver function (bilirubin ≤ 1.5 mg/dl), and adequate renal function (creatinine <1.5 mg/dl).

Treatment Plan: (Include dose adjustment) (Twenty lines not to exceed 75 characters per line)

1. The study will be an open-label upward dose ranging study for adenovirus-p53 vector (Ad5CMV-p53) in two patient groups. The two groups of patients will consist of a) resectable and b) non-resectable recurrent disease. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive on intratumor injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU).
2. Dose Escalation: The adenovirus dose will increase in one $\log_{10}$ increments for each group until 10^9 PFUs are reached. Patients entered at a given dose level will not be eligible for dose escalation. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients in each group (resectable and non-resectable).
3. All patients shall be registered with the Data Management Office
4. Patients with local-regional tumors will have injection of a total dose of 10 ml for tumors ≥ 4 cm in diameter or 3 ml for tumor <4 cm in diameter of the adenovirus preparation with the appropriate number of viral particles at multiple sites percutaneously or transorally. The treatment will be repeated three times weekly for two weeks. Dose escalation may proceed after a minimum two week follow-up of the last patient entered into the previous dose level. Treatment will continue on a monthly basis as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.
5. Those patients with surgically resectable disease will be treated by tumoral injection of adenovirus preparation as described above. The treatment will be repeated for two consecutive courses. Within four days of completion of the second course, the patients will be eligible to proceed with surgical resection. Prior to surgical closure, 10 ml of adenovirus preparation will be administered into the surgical defect (operative bed) and allowed to remain in contact for 60 minutes. The wounds are then closed and drains placed.

5.3 Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitation imposed by production of the adenovirus).

5.4 Dose Escalation: The adenovirus dose will increase in one-$\log_{10}$ increments for each group until 10^9 PFUs are reached. Patients entered at a given dose level will not be eligible for dose escalation. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients in each group (resectable and non-resectable).

5.5 All patients shall be registered with the Data Management office at ___. At the time of registration, a prestudy form shall be necessary on each patient. When applicable, information pertaining to important prognostic factors such as tumor histology, pretreatment weight loss, performance status, disease stage and extent, and prior therapy will be requested.

5.6 Patients with local-regional tumors will have injection of a total dose of 10 ml for tumors ≥4 cm in diameter or 3 ml for tumor <4 cm in diameter of the adenovirus preparation with the appropriate number of viral particles at multiple sites percutaneously or transorally. Direct endoscopic injections may also be used is required. Injections will be placed at approximately 1 centimeter increments.

5.7 The treatment will be repeated three times weekly for two weeks. Dose escalation may proceed after a minimum two week follow-up of the last patient entered into the previous dose level. Treatment will continue on a monthly basis as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.

5.8 Patients will wear a surgical mask for 24 hours following injection of the adenovirus. All medical personnel will wear masks and face shields routinely during endoscopy and injection of the adenovirus. Anti-tissves will be prescribed as necessary. All patients will be kept in isolation during the time they are receiving injections of the adenovirus and for 48 hours after the last injection.

5.9 Those patients with surgically resectable disease will be treated by tumoral injection of adenovirus preparation as described in 5.6 and 5.7. The treatment will be repeated for two consecutive courses. Within four days of completion of the second course, the patients will be eligible to proceed with surgical resection. At the completion of surgical resection, prior to closure, 10ml of adenovirus preparation will be administered into the surgical defect (operative bed) and allowed to remain in contact for 60 minutes. The wounds are then closed and drains placed. Post-operatively, on the third post-operative day (prior to drain removal), 10ml of adenovirus preparation is sterily introduced into the drains and retrograde placed into the wounds and is allowed to remain for two hours. The drains are then replaced to suction and removed when indicated by the attending staff surgeon.

6.0 PRE-TREATMENT EVALUATION

6.1 A complete history and physical to include performance status, recent weight loss, usual weight and concurrent non-malignant disease and its therapy, and all prior anticancer treatments must be recorded.

6.2 Laboratory studies shall include quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, HIV analysis, urinalysis, and chest x-ray.

6.3 Any residual toxicity from prior therapies should be recorded using the grading schema in Appendix C.

6.4 Appropriate studies should be obtained to fully define the extent and severity of existing or suspected malignant and non-malignant disease.

6.5 Measurements of disease that can be measured on a C.T. scan of the head and neck will be documented. The location and size must be recorded prior to treatment. A photograph of the area

The University of Texas
M. D. ANDERSON
CANCER CENTER

MEMORANDUM

DATE: October 4, 1995

TO: Dr. Gary Clayman
Department of Head and Neck Surgery

FROM: Myriam Brena
Secretary, Surveillance Committee (IRB)
Office of Protocol Research

SUBJECT: Administrative Approval of Revised Protocol HNS 94-001*, entitled "Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"

Official Approval Date: 10/2/95

Dr. Aman U. Buzdar, Surveillance Committee Chairman, reviewed and administratively approved the revision of the above named and numbered protocol.

This approval does not alter or otherwise change the annual review date of this protocol.

* Revision date 9/25/95

c: Leonard A. Zwelling, M.D.

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

M E M O R A N D U M

TO: Gary Clayman, M.D.
Department of Head and Neck Surgery

FROM: Leonard A. Zwelling, M.D.
Associate Vice President for Clinical
and Translational Research

DATE: October 10, 1995

SUBJECT: Activation and Distribution of New Protocol HNS 94-001

Attached find a copy of the above referenced protocol. This study is now ready for patient accrual. Should you have any questions, please do not hesitate to contact me.

Please distribute a copy of this protocol to the appropriate individuals in your department/section and collaborators.

LAZ/lmb
attachment

SURVEILLANCE COMMITTEE (IRB)
ANNUAL REVIEW OF CLINICAL, LABORATORY AND MISCELLANEOUS PROTOCOLS

9

REPORT DATE: DUE DATE: 08/31/95

.he annual review process is a requirement of this institution in compliance with federal regulations. The Study Chairperson is responsible for updating the protocol status each year by providing the following information to the Surveillance Committe (BOX 38) for potential approval by the due date shown above. Direct your questions to Myriam Brena, Secretary, Surveillance Committee (IRB) at extension 2-2933.

Protocol Number and Title: HNS 94-001 - "Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wildtype p53"

Study Chairperson: Dr. Gary Clayman
Department of Head and Neck Surgery

For Committee Review on: 09/20/95
Last Annual Review: / /

Does the protocol involve drugs or devices obtained via an Investigational New Drug Exemption (IND) or Investigational Device Exemption (IDE)? ☐ Yes ☐ No Number_____

Does this study include any products manufactured or produced at MDACC? ☐ Yes ☐ No
If yes, identify the room where the product is manufactured. _____

RECEIVED
SEP 29 1995
Office of Protocol Research

Current Protocol Status (Date)

_____ **Original IRB Approval**
Informed Consent (last revision) **Please attach the consent form with the latest revision of 2/16/94**
_____ **Not Yet Activated** (i.e. pending approval, drug, etc., but anticipated future activation).
(NOTE: Justification is required for any study not activated within one year (12 months) of IRB approval.
10/2/95 **Active** (study in progress and accruing patients).
_____ **Closed To New Patient Entry** (no new patient accrual, but patients continuing on treatment or still alive for follow up).
Date last patient entered _____
_____ **Terminated** (no activity - all patients off study). NOTE: A final report is required.
_____ **Withdrawn** (study never activated, no patient registrations. No future activity is anticipated)

Maximum number of patients or samples approved 42 Total accrual to date 0

Estimated number of additional patients or samples required to complete study _____

Have any recent reports of preliminary analyses been prepared since the last annual review? ☐ Yes ☐ No
If yes, provide a copy.

Total ADRs reported ✓ ADRs since last review ____ Number of treatment related deaths ____

Please provide brief synopsis of status of study including response and toxicity information (use additional pages if required)

Awaiting NIH/RAC approval prior to study commencing. Presently FDA approved

Study Chairman (Signature and Date)

Division Head or Department Chairman (Signature and Date)

Surveillance Committee Comments _____

Surveillance Committee Chairman or Designee (Signature and Date) 10-16-95

☐ Approved ☐ Deferred ☐ Approved Contingent

Rev. 8/94

10

INTROGEN
Therapeutics, Inc.

MEMORADUM

DATE: July 29, 1997

TO: Elias Dayam
Investigational Pharmacy
M.D. Anderson Cancer Center

FROM: Amber Hutchison
Sr. Clinical Research Associate AH

PROTOCOL: INT-002 (HNS 94-001)

RE: Study Close-out Drug Accountability and Material Return

Cc: Gary Clayman, M.D.
Patty Bruso
Jay Merritt, M.D.
Dee Connors
Study file

Enrollment and treatment of patients in the above clinical study has been completed. According to the Audit Report sent to me on July 28, 1997 there is one remaining vial in the freezer.

Attached is a Material Return and Disposition Record to be completed and returned with the shipment of one (1) vial of Lot 0057-0003 Part 8.50013A ($2x10^{10}$ PFU). This shipment should be sent to the attention of

If you have any questions, please do not hesitate to contact me at pager at ; or by